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DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

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1288

RECOMBINANT DNA ADVISORY COMMITTEE

MINUTES OF MEETING[1]

September 29, 1986

The Recombinant DNA Advisory Committee (RAC) was convened for its thirty-fifth meeting at 9:00 a.m., on September 29, 1986, in Building 31, Conference Room 6, National Institutes of Health, 9000 Rockville Pike, Bethesda, Maryland 20892. Mr. Robert Mitchell (Chair), Attorney at Law in California, presided. In accordance with Public Law 92-463, the meeting was open to the public. The following were present for all or part of the meeting:

Committee members:

Barbara Bowman
Royston Clowes
Mitchell Cohen
Bernard Davis
Charles Epstein
Susan Gottesman
Irving Johnson
Edward Korwek
John McGonigle
Robert Mitchell
Gerald Musgrave
Paul Neiman
Joseph Pagano
Thomas Pirone
David Pramer
Fred Rapp
Jeffrey Roberts
Frances Sharples
Anne Vidaver
LeRoy Walters
Anne Witherby
William J. Gartland, Jr. (Executive Secretary)

A committee roster is attached (Attachment I)

Ad hoc consultant:

Gerard McGarrity, Coriell Institute for Medical Research

Non-voting agency representatives:

Joel M. Dalrymple, Department of Defense
George Duda, Department of Energy
Bernard Greifer, Department of Commerce
Phillip Harriman, National Science Foundation
Morris A. Levin, Environmental Protection Agency
Henry I. Miller, Food and Drug Administration
George P. Shibley, Department of Agriculture
Sue A. Tolin, Department of Agriculture

[1]The RAC is advisory to the National Institutes of Health (NIH), and its recommendations should not be considered as final or accepted. The Office of Recombinant DNA Activities should be consulted for NIH policy on specific issues.

Liaison representative:

Daniel P. Jones, National Endowment for the Humanities

National Institutes of Health staff:

W. French Anderson, NHLBI
Stanley Barban, NIAID
Irving Delappe, NIAID
Rosalind Gray, OD
Becky Lawson, NIAID
Rachel Levinson, OD
Bernard Talbot, NIAID
Janine Trempy, NCI
Carol Y. Wigglesworth, OD

Others:

Joseph Autry, NIMH
Frederick S. Betz, Environmental Protection Agency
Irene Brandt, Eli Lilly and Company
Chia Ting Chen, Department of Labor
Barbara Culliton, Science Magazine
Peter Farnham, American Society of Biological Chemists
Richard Frankel, General Accounting Office
Andreas Freudenberg, German Marshal Fund
Julie Gage, Kennedy Institute
Jeff Gibbs, Association of Biotechnology Companies
Alan R. Goldhammer, Industrial Biotechnology Association
Colin Gracey, Committee for Responsible Genetics
Ann Huang, Environmental Protection Agency
Dorothy Jessup, Department of Agriculture
Roger S. Johnson, Genetic Engineering News
Rosamond Katz, General Accounting Office
Martha Ladenheim
Eckhard Lieb, German Marshal Fund
David Long
Mary Jane Potash, National Academy of Sciences
Cheryl Martin, Blue Sheet
James H. Maryanski, Food and Drug Administration
Elizabeth A. Milewski, Environmental Protection Agency
David Moore, Association of American Medical Colleges
Stuart Newman, Committee for Responsible Genetics
Robert B. Nicholas, Blum, Nash, & Railsback
Elliott A. Norse, Ecological Society of America
Greg Pearson, Blue Sheet
Harvey S. Price
Kevin O'Connor, Office of Technology Assessment
Richard Raffa, Sandoz Corporation
Jane Rissler, Environmental Protection Agency
Rex Rhein, McGraw-Hill World News
Mark Rhodes, National Science Foundation
Marvin Rogul, The Rogul Group

Harold M. Schmeck, New York Times
Mark C. Segal, Environmental Protection Agency
Janet Shoemaker, American Society for Microbiology
Marvin Stodolsky, Department of Energy
Clarence E. Styron, Monsanto Company
Charles Turbyville, NIH Week
Robert Wachbroit, University of Maryland
Joseph Van Houten, Schering-Plough
William J. Walsh, National Academy of Sciences
Susan Walton, ASM News
Charles Weiner, Massachusetts Institute of Technology
Roberta Weiner, Committee for Responsible Genetics
John Whalen, National Institute for Occupational Safety and Health
Nachama L. Wilker, Committee for Responsible Genetics

I. CALL TO ORDER AND OPENING REMARKS

Mr. Mitchell, Chair, called the September 29, 1986, meeting of the Recombinant DNA Advisory Committee (RAC) to order. He stated that the meeting had been publicly announced in the Federal Register on June 25, 1986 and August 15, 1986, in conformity with law and that the meeting is open to the public.

Dr. Gartland informed Mr. Mitchell that a quorum was present for the September 29, 1986, meeting. Mr. Mitchell stated that there were three major actions on the meeting agenda and that in chairing the meeting his intention was to discuss items in the order they appeared on the agenda and to attempt to maintain time estimates as stated in the agenda for each item. The major items were noted as Agenda Items IV, V, and VI, and Mr. Mitchell underlined the point that all three had been previously published in the Federal Register.

Mr. Mitchell went on to state that he would recognize speakers in the following order: primary reviewers; other RAC members; ad hoc consultants; non-voting representatives to the RAC; RAC's administrative staff; members of the public who submitted written documents or comments; and finally other members of the public who may wish to comment. He then stated that comments may be submitted after the meeting and that these comments may be used to assist the Director, NIH, in arriving at a decision on any agenda item.

Mr. Mitchell then introduced Dr. Paul Neiman, a new member of RAC, who was making his first appearance at a RAC meeting. Dr. Neiman is from the Fred Hutchinson Cancer Research Center in Seattle, Washington. He also welcomed back Dr. McGonigle, who had been ill for a period. He then turned to Dr. Gerard McGarrity, previous Chairman of the Working Group on Release into the Environment, and now Chairman of the Working Group on Definitions, to announce that he was serving the RAC at this meeting as an ad hoc consultant. He also welcomed Dr. Elizabeth Milewski, an ex-member of the RAC staff, who is now working for the Environmental Protection Agency.

II. MINUTES OF THE JANUARY 27, 1986, RAC MEETING

Mr. Mitchell called on Dr. Musgrave to review the minutes (tab 1274) of the January 27, 1986, meeting of the RAC. Dr. Musgrave stated that he had reviewed the minutes and found that they appeared to be correct .

Dr. Musgrave moved the minutes be approved as they appear in tab 1274. Dr. Walters seconded the motion. Mr. Mitchell called for any other additions, deletions or corrections, and hearing none put the motion to a vote. The motion to approve the minutes as appearing in tab 1274 was unanimously approved.

III. REPORT OF THE WORKING GROUP ON DEFINITIONS

Mr. Mitchell called on Dr. McGarrity to present the report (tab 1280) of the Working Group on Definitions.

Dr. McGarrity stated the Working Group on Definitions is a new group, formed this summer, made up predominantly of former and present members of RAC and that many of this group had also been a part of the Working Group on Release into the Environment. The charge that had been put to the group was to consider the current definitions of "recombinant DNA" and "deliberate release into the environment", as used in the NIH Guidelines for Recombinant DNA Research.

Dr. McGarrity then stated that since the working groups were advisory to RAC, the RAC would have the following options in regard to the recommendations he would be

presenting:

1. Reject them outright, saying that they are not needed or they are bad or not worthy of consideration.

2. Accept the definitions completely, as presented, and in that case they would have to be published in the Federal Register for public comment and re-presented to RAC for a vote at the next meeting.

3. Accept the notion and spirit of the recommendations but suggest further refinement. The Working Goup could then meet again and publish its revised recommendations in the Federal Register and present them at the next RAC meeting.

Dr. McGarrity then pointed out that the present definition of "recombinant DNA" can be found in the May 7, 1986, issue of the Federal Register on page 16959, in Section I-B of the NIH Guidelines. He stated the Working Group had considerable discussion on this definition but that there was some concern about the mechanics of attempting to define such a rapidly changing and still-developing specialty. Therefore, the Working Group decided not to change the definition, but rather that the following sentence be added after the first paragraph of Section I-B, to read as follows:

> "Genomes which contain only deletions, single base changes or rearrangements are not considered to be recombinant DNA, irrespective of the method by which they were produced."

Dr. McGarrity stated that the Working Group had passed this resolution by a vote of 11 in favor, no opposed and 2 abstentions. He further amplified that the sense of the Working Group was that this sentence is intended to cover duplications, amplifications, and translocations, but is not intended to cover movement of plasmid or viral DNA into a chromosome.

In relation to the definition of "deliberate release into the environment," Dr. McGarrity stated that the RAC will be considering a proposal from Susan Gottesman to amend Section III-A-2 of the Guidelines. At present this section contains the words, "except certain plants as described in Appendix L." Dr. Gottesman's proposal is that two new sentences be added, i.e., "Deletion derivatives not otherwise covered by the Guidelines," and "Organisms covered in exemption III-D-2." He further noted that the Working Group endorsed both of these proposals by a wide margin.

Dr. McGarrity said that the Working Group struggled for many hours before voting that the following sentence be added at the end of Section III-A-2:

> "The term 'deliberate release' is defined as a planned introduction of recombinant DNA-containing microorganisms, plants, or animals into the environment."

Dr. McGarrity stated that the vote on this issue was very close: 5 in favor, 4 opposed and one abstention.

The Working Group moved that Section III-A-2 of the NIH Guidelines be amended to read as follows:

> "Deliberate release into the environment of any organism containing recombinant DNA except small-scale field tests

in which there is adequate evidence of biological and/or physical control of the recombinant DNA-containing organisms. The nature of such evidence is described in Appendix L, M, and N.

Dr. McGarrity stated that this language was approved by the Working Group by a vote of 10 in favor, 1 opposed and no abstentions. Appendix L would be the current Appendix L dealing with plants with future changes to be determined by the RAC. Appendices M and N would be parallel sections, to be written, covering animals and microorganisms.

Dr. McGarrity then said that the members of the Working Group discussed the possibility of holding a workshop to address issues of environmental release. A motion was made, but defeated, to request the National Research Council (NRC) to hold such a workshop to involve various Government agencies. The defeat of the motion centered around the view in the Working Group that the NIH could better serve its needs for scientific advice in this area by directly calling together scientific workgroups rather than asking the NRC to do so.

In summary, Dr. McGarrity stated the recommendations that the Working Group was putting forward are simply extensions of the philosophy of revision of the Guidelines, as has been set out previously, to conform with new applications and experience in the field. He stated that he had just attended a decennial review conference on cell and molecular biology of cell cultures, and that the previous meeting, 10 years ago, had stressed research on monoclonal antibodies and growth factors. This year the emphasis was on gene expression, recombinant DNA technology, and oncogenes. He mentioned the great advances in research on monoclonal antibodies and growth factors that have taken place in the past decade; if recombinant DNA experiences a similar growth in the next decade, the Guidelines will have to be very flexible to keep up with these changes.

Dr. Gottesman stated that she felt Dr. McGarrity had presented the sense of the Working Group meeting very well but that he had left out some things which the Working Group had spent a lot of time discussing. She stated the Working Group felt the term "deliberate release" unfortunately had a "nasty" connotation, but that in the end no better alternative phrase could be agreed upon.

Dr. Gottesman explained that the issue of the definition of "recombinant DNA" was discussed by the Working Group in the case where pieces of DNA are spliced together in vitro, but no "foreign" sequences are added. The current Guidelines still define this as recombinant DNA because the current definition says nothing about where the molecules come from; as long as the molecules are "constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate," they are considered to be recombinant DNA.

Dr. Gottesman stated that we have not had to face this issue in the past because self-cloning or rearrangements involving an organism and the organisms that normally exchange DNA with it, have been exempt from overview for laboratory experimentation under the Guidelines. But now as we consider human gene therapy or deliberate release into the environment the question is whether it is appropriate to cover in the Guidelines organisms in which recombinant DNA has been used in their construction but they are not recombinant in the sense of having foreign DNA in them. The new proposed definition would be one way of pulling this set of organisms out of the Guidelines entirely. An alternate way of dealing with this is not to change the definition of recombinant DNA, but to deal with this set of organisms elsewhere in the Guidelines.

Dr. Gottesman then brought up the issue of deliberate release by questioning the semantics of the term "release." Does this just mean there is no roof? Does it require establishment of the organisms in the environment? Does "deliberate" mean that the

release had to be planned to constitute "deliberate release"? If "deliberate release" were defined very narrowly it could produce an either/or situation whereby if you weren't covered by the deliberate release definition, you could come under Section III-D of the Guidelines, "Exempt Experiments."

Dr. Gottesman said she favored setting up additional categories under Section III-A-2 of the Guidelines which would be similar to Appendix L of the Guidelines in which approval could be given by a subcommittee of the RAC for certain defined types of "deliberate release" experiments without having to have them go through the Federal Register notice procedure and coming before the full RAC. Further, she stated, that RAC should consider setting up working groups to start to write these new Appendices modelled after Appendix L.

Dr. McGarrity then stated that the Working Group on Release into the Environment has previously drafted a "Points to Consider" document for the introduction of micoorganisms into the environment and that this could be a starting point for a new Appendix M.

In response to a question from Dr. Pramer, Dr. Gartland noted that Appendix L has never been used to approve a proposal. Dr. Talbot stated that a proposal was submitted under Appendix L but it was determined that it did not meet the criteria in Appendix L.

Dr. Clowes said that he believed the conclusions of the Working Group were most valuable. Currently Section I-B of the Guidelines states:

> "In the context of these Guidelines recombinant DNA molecules are defined as either (1) molecules which are constructed outside living cells..."

Dr. Clowes felt the wording should read:

> "In the context of these Guidelines recombinant DNA molecules are defined as either: (1) *recombinant* molecules which are constructed outside living cells..."

He believed only new combinations from different organisms should be covered and not deletions, single base changes, rearrangements, et cetera. He also stated that "deliberate release" had a perjorative connnotation of something dangerous and that he preferred deleting this phrase wherever it appeared in the Guidelines and substituting the phrase "planned introduction", which he said means the same thing.

Dr. McGarrity reiterated that many substitute phrases were discussed in the Working Group and that there was no phrase which was acceptable to everyone around the table. Dr. Gottesman stated that it was not clear that "planned introduction" meant the same to everyone as "deliberate release," and that since "deliberate release" had been the term previously used, there was virtue in leaving this term in place, despite the negative connotation. Dr. Gottesman also felt that Dr. Clowes' proposal to insert the word "recombinant" in Section I-B involved a similar problem.

Dr. Rapp stated many people in the field regarded "recombinant DNA" as referring to any case in which recombinant DNA technology was used, including deletions, rearrangements, et cetera, within an organism or between organisms. He also stated that a change in definition to eliminate such from coverage is a big step and not a trivial point.

Dr. Davis said RAC's purpose is to protect from harm. Activities which go on in nature, such as gene deletions and rearrangements, cannot be controlled by RAC. Similarly, he stated that he felt the problem in defining "deliberate release" is seeking a scientific

solution for a non-scientific problem. He noted that the ability of an organism to become established in the environment depends entirely on the properties of the organism in competition with everything else in the environment and not on numbers of organisms released, and that if an organism is not competitive, despite local, transient effects, no widespread, global effects are to be anticipated from such releases. He cited the example of the ice-minus organism previously reviewed by RAC, and said that removing a gene from an organism producing a phenotype that already exists in nature is not dangerous.

Dr. Korwek said he was concerned that RAC's attempts at redefining these terms could have impact outside the NIH in that the Office of Science and Technology Policy is working on a regulatory scheme for biotechnology, and that redefinition by NIH could have a "ripple effect" on the regulatory agencies leading to profound regulatory implications.

Dr. Talbot replied that the RAC's charge is to recommend to the NIH Director changes in the NIH Guidelines for Recombinant DNA Research. The NIH Director is a member of the Biotechnology Science Coordinating Committee where he can coordinate NIH policies with the policies of the regulatory agencies.

Dr. Gottesman noted the RAC deals only with recombinant DNA, while EPA deals with broader aspects of biotechnology. RAC should deal with its charge, without worrying about implications vis-a-vis the concerns of the regulatory agencies.

Dr. Korwek then questioned Dr. McGarrity as to the Working Group proposed definition of "recombinant DNA." He was bothered by the phrase in the Working Group minutes that, "It was the sense of the Working Group that this is not intended to cover movement of plasmid or virus DNA to a chromosome..." He stated that a definition should stand alone, without interpretive statements. A definition that is not self-sufficient to state clearly what is meant is not a good definition. He also said he did not understand the proposal to add the sentence concerning planned introduction to the "deliberate release" definition. If a release is not planned, how can it be controlled under the Guidelines?

Dr. Gottesman explained that the Working Group had been trying to come up with a term to replace "deliberate release" because of its negative connotations of just releasing something into the environment and not caring about what becomes of it and not controlling it; whereas "planned introduction" seemed to suggest more positive feelings in that it seemed to connote a purposeful introduction and more control afterwards. She said she personally however saw almost as much problem with the phrase "planned introduction" as with the phrase "deliberate release" in terms of what people mean by the term. If a person proposed to do a field test in which they plan an introduction then it clearly would be covered by the new definition. However, if they simply decided to dump material into the environment without caring what happens to it, it might be claimed that it does not fall under the definition of "planned introduction," and therefore is out from under the control of the Guidelines.

Dr. Rapp argued that just because something occurs in nature does not mean we should introduce it into a population. Dr. Cohen said deletions or point mutations in a higher animal might involve different considerations than in a microorganism.

Mr. Mitchell noted that the changes in the Guidelines proposed by the Working Group had not been published in the Federal Register and therefore RAC could not take final action at this meeting. However, RAC, if it desired, could take a vote on the matter which could then be published for comment and reconsideration at the next meeting, or, if desired, could recommit the matter back to the Working Group for further review in light of the discussions which had taken place at today's meeting.

Dr. Neiman stated there were differences between microorganisms which undergo rapid and frequent genetic change, and more complex organisms where a simple rearrangement could produce a highly deleterious event. Despite being sympathetic to efforts to avoid unnecessary over-regulation, he was concerned about non-supervision of certain experiments. Dr. Davis agreed.

Dr. Sharples then made the following motion:

> "That we refer this matter back to the Working Group on Definitions to take account of the discussion that we've had here this morning and perhaps make some modifications in what the Working Group presents at the next RAC meeting."

Dr. Talbot asked for clarification as to whether Dr. Sharples was referring to the definition of "recombinant DNA" or to all the recommendations of the Working Group. Dr. Sharples stated that her motion was meant to refer to all of the recommendations. Dr. Korwek seconded the motion and Mr. Mitchell called for discussion on the motion.

Dr. Clowes asked if the recommendations that the Working Group will come up with would have to come back to the RAC first for further comment or whether they could be clarified in such a way as to have them placed in the Federal Register prior to the next RAC meeting so that a vote could be taken on them at the next meeting.

Mr. Mitchell stated he believed it would depend upon how precise the recommendations would be and that it might be better to have further discussion by the RAC before publication in the Federal Register.

Ms. Witherby suggested it would be helpful if the members of RAC could get the new report from the Working Group prior to the next meeting so it could be studied in advance of the meeting. Mr. Mitchell replied that this would have been the case this time except that the meeting was held on September 5th and that there hadn't been enough time between then and today to accomplish this.

Dr. Gottesman stated she felt that in order for the motion to be really productive it would be useful if the Working Group recommendations were published in the Federal Register and comments from the public sought prior to the next meeting so that a vote could be taken at that time. She added that some of the recommendations of the Working Group were not as controversial as the definition changes. She offered to amend Dr. Sharples' motion to include a request that the Working Group proposals be published in the Federal Register prior to the next RAC meeting.

Mr. Mitchell suggested that rather than making that part of the motion, that it could be a matter the Working Group could determine itself once they met and determined what progress they had made.

Dr. McGarrity agreed with Dr. Gottesman's suggestion of Federal Register publication before the RAC meeting, as it would not only give RAC members more time to look over the new recommendations but it would be put before a broader audience. Dr. Johnson agreed.

Dr. Sharples agreed to incorporate into her motion the concept that the new Working Group recommendations would be published in the Federal Register if the meetings were held in time and the recomendations were clear enough.

Dr. Davis requested that the Working Group consider replacing "deliberate release" with the phrase "deliberate introduction" because of the problem of definition of the

word "planned."

Mr. Mitchell took a vote on Dr. Sharples' motion to recommit the recommendations back to the Working Group for further discussion and clarification and to have their new recommendations published in the Federal Register, if possible, prior to the next RAC meeting.

The motion was passed unanimously with a vote of twenty in favor, none opposed and no abstentions.

Dr. Sharples requested that the Working Group on Definitions be expanded to include additional RAC members who had participated in the discussion today. It was agreed to do so.

IV. PROPOSED AMENDMENT OF SECTION III-A-2

Mr. Mitchell called on Dr. Gottesman to begin review of the proposed amendment of Section III-A-2 of the Guidelines (Tabs 1264, 1269/I, 1281).

Dr. Gottesman said, since she had proposed the amendment, she wished to explain what it does and why she proposed it. Basically, the proposal would change the current Guidelines concerning some classes of deliberate release experiments so that they would no longer come before the RAC in any form, but instead be treated the way laboratory experiments of this class are currently treated. Currently there are certain types of laboratory experiments which are exempt from review under the Guidelines, as for instance experiments involving rearrangement of a genome, i.e., "self-cloning," and transfer of DNA from one organism to another when those organisms naturally exchange genetic information. However, the Guidelines do not exempt these type of experiments where there is deliberate release of such recombinant DNA-containing organisms into the environment.

Dr. Gottesman explained that among the types of deliberate release experiments which would be exempted from review by this proposal the first would be "deletion derivatives not otherwise covered" in the Guidelines.

The second category of deliberate release experiments to be no longer under the Guidelines would be "organisms covered in Section III-D-2," i.e., rearrangements within a single non-chromosomal or viral DNA source only. She cited the example of an experiment which rearranges the DNA of a plasmid, puts it back into an organism, and deliberately releases it into the environment which would no longer be covered by the Guidelines. She noted that the amendment would not change coverage under the Guidelines for experiments where rearrangement of the chromosome of bacteria or any other organism had taken place, but only change coverage under the Guidelines for deletions and rearrangements within non-chromosomal DNA sources, i.e., plasmids or viruses.

Dr. Gottesman explained that her reasons for proposing the amendment are based on her view that the Guidelines were only meant to cover "unique organisms." The previous exemption for laboratory experimentation of classes of organisms which were prepared using recombinant DNA but which are not really should now be extended to deliberate release of such organisms.

Dr. Gottesman stated that she believed this amendment is a continuation of the effort already begun in Appendix L to classify deliberate release of certain organisms which do not require special review by the RAC. She further stated that she has also proposed to the Working Group on Definitions a further step in this direction with the preparation of new Appendices M and N, parallel to Appendix L.

Since deletions occur in nature, deletions made in the laboratory will not result in the creation of unique organisms. These types of experiments should be exempted from the Guidelines as well as experiments which result in DNA rearrangements within a single, non-chromosomal or viral source.

Dr. Gottesman stated it is very important to realize that this proposal does not guarantee that every deletion is without effect or that every deletion will not change the behavior of the organism. However, she submitted that since deletions are not "new" they should not be considered by the RAC. Further, it is not meant to imply that some deletions should not be regulated by an agency of the Government, but simply not by the NIH. For example, she stated, a deletion in a pathogen which would be contemplated for release into the environment would certainly fall under the regulatory purview of the Environmental Protection Agency.

Dr. Roberts stated he agreed with the proposal as a cautious step forward in relieving unnecessary regulation, in that he felt these types of organisms occur naturally and could be made by traditional genetic means other than recombinant DNA technology and thereby do not need to be covered by the Guidelines.

Dr. Sharples stated that for several reasons she disagreed with the proposal by Dr. Gottesman. She stated she did not believe it was possible to make an a priori judgment that a deletion or rearrangement would not result in a negative environmental effect. She further stated that for any kind of genetic modification it is important to understand how the modification will alter the behavior and relationship of the organism in the environment. To find out if and how a deletion or rearrangement is translated into an environmental change, you have to go and look for the answer. The answer does not present itself merely from knowing that all you have done is deleted a tiny bit of DNA from an organism's genome; rather, getting the answer requires that some work be done and that some scrutiny be applied. As an example, she cited the RAC's deliberations on the ice-minus bacterium where the removal of a gene for production of an ice-nucleating protein led to a shift in the relationship of the bacterium with the ambient environment. Although the change in the relationship in this instance is not very likely to result in further negative effects, it cannot be denied that a change in the organism's role and behavior did occur because of the removal of a single gene.

Dr. Sharples also cited other work on the relationshp between genes and virulence in *Agrobacterium tumefaciens*. It was found that in grape vines resistance to *Agrobacterium* infection is the result of a hypersensitivity response by the plant to a bacterial gene. When this gene is deleted from the bacterium, the plant no longer resists the infection and this results in tumors in the vines associated with crown gall disease. This genetically determined shift in the host range of the bacterium could not have been predicted; it had to be looked for and established by examining the specifics of the situation. We know from these examples that single gene deletions, however minor the genetic change they entail, can translate into changes in environmental relationships and you will not find out what those changes are and whether they are harmful or not until you look for them.

Dr. Sharples said she believed that RAC should continue its oversight oF organisms for environmental release as is now required regardless of the nature of the genetic change they have undergone to ensure that investigators who propose field tests in fact have considered the potential for the genetic change they have made to translate into significant environmental differences.

Dr. Sharples said the RAC was not burdened by its present workload in oversight of environmental release experiments. She pointed out that RAC has not recently received any proposals to conduct field tests and that the "Points to Consider" for

environmental release has never been used. She stated further her concern that this proposed amendment would give a false impression that these organisms do not need review by any group and that the amendment could also lead other agencies to adopt a similar attitude.

Finally, Dr. Sharples stated she viewed the proposed amendment as an extension of Dr. Gottesman's view of what constitutes "recombinant DNA", a view which is based on product rather than process. She stated that she felt this may be contrary to the purpose of RAC which exists not to regulate products but to ensure that recombinant DNA research is conducted safely. If the research leading to production of particular organisms involves the use of recombinant DNA techniques, then Dr. Sharples believed RAC should have jurisdiction over that research. If field testing of that organism is part of the research program then the RAC's oversight should extend to the field testing. Amending the Guidelines in accordance with Dr. Gottesman's proposal may lead environmental intervenors to initiate further litigation.

Dr. Vidaver said that she agreed with Dr. Gottesman's proposal. She concurred with Dr. Sharples that the purpose of the RAC is to review a process. However, she added, she does not feel this precludes the RAC from reviewing the product. Any deletion or rearrangement can have an effect. But in the ice-minus and *Agrobacterium tumefaciens* cases, there are already comparable conditions in nature. She did not feel it necessary for the RAC to review deletions or rearrangements since perhaps 99 percent of these cases would not be of interest to the RAC and the probability of such organisms having an adverse effect on the environment was minimal.

Dr. Johnson agreed strongly with Dr. Sharples that RAC should continue scientific oversight; however the Guidelines should be modified when it makes sense to do so. He stated that EPA would still be have to be notified of and/or review any deliberate release experiments and that therefore any such experiment would still be regulated in terms of product.

Dr. Gottesman then made the following motion:

> "That the RAC accept the proposal to amend Section III-A-2 of the Guidelines to read:
> 'Deliberate release into the environment of any organism containing recombinant DNA, except:
>
> 'a. Certain plants as described in Appendix L.
> 'b. Deletion derivatives not otherwise covered by these Guidelines.
> 'c. Organisms covered in exemption III-D-2.'"

After Dr. Johnson seconded the motion, the Chair called for further discussion.

Dr. Gottesman replied to Dr. Sharples with the statement that she felt that RAC's looking at the recombinant DNA process was appropriate. However it is important that the RAC does not give the impression that an experiment is necessarily "special" just because recombinant DNA was used. RAC will lose scientific credibility if it maintains "that because recombinant DNA was passed magically over an organism that it does something special to it." She underlined the fact that deletions and rearrangements take place in nature, and just because recombinant DNA techniques are used to elicit these same genetic changes, RAC should not be reviewing them as unique and special cases.

Dr. Gottesman said she was pleased that other government agencies will be reviewing those organisms which need to be looked at for environmental effects. However, on the other hand, she hoped that they won't review them in extra detail just because

recombinant DNA was used to make them.

Dr. Korwek noted that the current Guidelines state that if a deliberate release experiment is submitted for review to another Federal agency, then the NIH Office of Recombinant DNA Activities may, "determine that such review serves the same purpose, and based on that determination, notify the submitter that no RAC review will take place, no NIH approval is necessary, and the experiment may proceed upon approval from the other Federal agency"; for such experiments, adoption of the Gottesman proposal could be viewed as RAC only giving up the right of first review. He asked if RAC still wanted to review those experiments that would not get review by another agency.

Dr. Cohen cited the example of using a chemical agent to make a deletion in a microorganism and asked what EPA constraints a researcher would be under to field test such an organism.

Dr. Elizabeth Milewski of the Environmental Protection Agency stated that EPA's policy is to look at all biotechnology products that are microorganisms which fall under the EPA's toxics and pesticide statutes. Because these statutes are product-oriented, the EPA would review organisms generated by chemical mutation, UV-irradiation, cell fusion, or biotechnology, including recombinant DNA. Under the toxics statutes, the EPA only covers research that is subsidized by industry, but under the pesticide statutes coverage is broader because EPA looks for the effects an organism would have on the environment and has little interest in the way the organism was generated. The hypothetical research as propounded by Dr. Cohen would have to get approval for small-scale field testing if it fell under the Federal Insecticide, Fungicide and Rodenticide Act, whether the investigator utilized recombinant DNA, chemical mutagenesis, or UV-mutagenesis to produce the test organism.

Dr. Roberts said that he felt RAC's attention should be saved for organisms which were developed using recombinant DNA that were unique and not simply engineered copies of organisms that could be found in nature. Dr. Clowes agreed.

Dr. Gottesman stated she felt that RAC should not continue jurisdiction over the types of organisms that would be removed from coverage by the Guidelines under her proposal because the same organisms could be produced using traditional genetic techniques and that merely the use of recombinant DNA technology to produce them does not make them unique.

Dr. Rapp agreed with Dr. Gottesman that the use of recombinant DNA techniques to engineer an organism that exists in nature does not make it unique. However he said the RAC was created to generate public confidence and he was concerned about "whittling away" the types of experiments covered by the Guidelines.

Dr. Sharples said that in the meeting of the Working Group on Definitions it became apparent that geneticists were using the term "rearrangement" to include duplications and therefore if the RAC supported the proposed amendment that this would mean it would not review organisms that are used to double, triple, quadruple or possibly increase by 100 times the production of a given protein and that this does not limit itself to moving one gene or changing the position of one gene relative to another, but also increasing the production of gene product.

Dr. Miller of the Food and Drug Administration stated he felt the RAC was to be commended for its tradition of timely and appropriate modifications to the Guidelines and that such actions have made RAC a benchmark for other groups and for regulation around the world. He cited the example of the evolution of the Guidelines in regard to most large-scale uses of recombinant organisms where cloning has been done in *B. subtilis*, *Saccharomyces* or *E. coli*, which has streamlined both research and

commercial applications of this technology.

Dr. Miller stated he supported the proposed amendment which continues this tradition. He said just because the RAC would exempt something from oversight does not by any means mean that all other Federal agencies would do the same. The FDA, he stated, has an extremely extensive and tight net of what is overseen and regulated. The ice-minus *Pseudomonas syringae*, which, under this proposal, would be exempt from oversight by RAChas been subjected to very substantial regulation outside the NIH. Recombinant DNA manipulated live attenuated vaccines would continue to be treated by the FDA the same way as those that are conventionally manipulated. USDA will do the same for animal vaccines. He added that his colleagues in the European Commission and in Japan who are involved in Governmental regulation were pleased to learn of this conservative, but important, step forward being considered by the RAC.

Dr. Pramer stated his support for the proposed amendment adding that if the RAC/NIH redefines recombinant DNA molecules in the way recommended by the Working Group on Definitions that it will, by that action, remove from its purview the very experiments under discussion in this proposal.

Dr. Davis said the definition of the recombinant DNA process should be used to delineate classes of unique and potentially dangerous products. He agreed with Dr. Gottesman that the RAC's scientific credibility was very important and at present was very high and should remain so. He stated that if RAC did not "continue to exhibit the flexibility to whittle away those things that are so obviously harmless we will lose that credibility."

Dr. Davis then questioned Dr. Sharples concerning her example of the *Agrobacterium* that has become more virulent as a result of the deletion of a particular gene. He said that in the medical field, which he knows much better than the plant field, that virulence and ability to produce epidemics are two different concepts and it's perfectly easy to isolate a variant of the diphtheria bacillus that produces several times as much toxin as the ones that are normally encountered, but they do not spread in nature. Their overall ability to survive is impaired, even though in an animal test they might be extremely virulent. He asked if there is any evidence that this *Agrobacterium* organism that has had a deletion that makes it more virulent has also gained an ability to spread.

Dr. Sharples replied that the only way to answer that question was to require an investigator to go and do an experiment to find out. Dr. Davis questioned, "But it is not found in nature?," and Dr. Sharples replied, "That's right."

Dr. Cohen inquired as to the use of the word "organisms" as opposed to "microorganisms" in the proposed amendment. Dr. Gottesman explained that the proposal is general in nature. At the moment, investigators are easily able to make deletions in microorganisms, viruses, and plasmids. For rearrangement where the word "organisms" appears, it specifically refers only to non-chromosomal or viral sources of DNA and does not cover even microorganism chromosomal DNA.

Mr. Elliott Norse, Director of Public Affairs for the Ecological Society of America, stated that there seems to be some disagreement about whether deletions and rearrangements were environmentally significant or not. It was his understanding that Dr. Davis felt they were trivial and that Dr. Gottesman felt that they may not be trivial but that this was irrelevant to the question at hand. Mr. Norse stated he believes they are not trivial; quantitative changes in the characteristics of organisms can affect their impacts on ecological systems and what was being discussed were things that have the potential to produce quantitative changes.

Mr. Norse said he agreed with Dr. Miller in one sense, and that is that what RAC does is very important in setting precedents for what other organizations do in this field. He

voiced concern that if RAC makes this decision, there will be pressure for other agencies to follow along. He stated that relaxation of the Guidelines in the case of laboratory experiments was "empirical," in that as it was discovered problems did not exist, the Guidelines were relaxed. However, he said that we haven't had those precedents as far as environmental releases of organisms, particularly microorganisms, are concerned. Until we get such a body of information it is premature to make the kind of proposal that Dr. Gottesman is making now, which may be entirely appropriate a year or two from now.

Dr. Pirone said there is no question that genetic changes can result in changes in host range and pathogenicity, but that is not the issue. He stated he supported Dr. Gottesman's proposal as an eminently logical and scientifically sound approach because it will exempt events that can and do occur naturally. If Dr. Gottesman's proposal were rejected, an "absurd logical" conclusion could be that we should go out into the environment and collect a wide range of naturally occurring biotypes of organisms and determine whether they have adverse effects on the environment and then attempt to ban them from nature.

Dr. Sharples stated that she did not believe that rejecting Dr. Gottesman's proposal would undermine the RAC's scientific credibility since these were the very Guidelines that contributed to the RAC's scientific credibility. She said that the RAC should also be concerned about its credibility with the lay public, and the public's perception of whether this technology is being dealt with safely and responsibly. She was concerned that if this proposal is accepted, then certain research applications will go without any review by any agency, and that would not be appropriate.

Mr. Mitchell mentioned a letter from Dr. John Moore of the EPA (tab 1281). This letter states that EPA is forming a Biotechnology Science Advisory Committee (BSAC), and requests that the RAC "consider postponing making a recommendation to the Director of the NIH concerning changes in the NIH Guidelines which would affect oversight of deliberate releases of microorganisms to the environment," and that "the RAC and the BSAC coordinate their efforts on the very difficult technical problems in the area of environmental release."

Dr. Gottesman stated that the concerns of the EPA were somewhat different from those of the RAC; what the RAC does, does not preclude EPA from doing what they want and that it is important that RAC vote on the proposal. She reminded the RAC that it is a body which is advisory to the Director of NIH and that any recommendations made by the RAC on this proposal would be just that and would not constitute final action. She said that the amendment does not state that deletions have no effects on organisms and that therefore no one needs to review them, but is simply saying that the NIH Guidelines should not make a special case of deliberate release into the environment of organisms which contain deletions merely because these deletions were accomplished by means of recombinant DNA technology.

Dr. Johnson agreed with Dr. Gottesman and stated that the RAC is advisory to the Director of NIH and that it is his prerogative then to coordinate with the EPA and that therefore the RAC should proceed to make a recommendation to the Director without awaiting any direction from the EPA. Dr. Clowes agreed that the RAC should take a position concerning this amendment so that other committees can have the advantage of knowing the RAC's arguments and the outcome of its deliberations.

Dr. Neiman stated that the deletion of the long arm of chromosome 6 of man, which occurs naturally, is associated with the activation of an oncogene which results in a high risk of T-cell lymphomas in individuals who inherit this trait. He asked Dr. Gottesman if a clinical experiment containing such a deletion would no longer be reviewed by the RAC under the proposed amendment. She explained that the proposal covers release into the environment only, and does not in any way change the

Guidelines in regard to recombinant DNA or DNA made from recombinant DNA used in human gene therapy.

Dr. Davis stated that, although he felt coordination with the EPA was desirable, RAC should act now. The Director of NIH could represent the RAC's position to the Biotechnology Science Coordinating Committee (BSCC). Dr. Pramer made the suggestion that perhaps some members of the EPA BSAC could be invited to participate in future meetings of RAC working groups.

Dr. Walters then stated, to avoid possible misinterpretation on the part of investigators who may look at Section III-A-2 of the Guidelines and think no other agency is concerned about deliberate release, that a footnote be added to this section stating that investigators considering deliberate release should consult the applicable sections of EPA regulations or specific statutes that govern EPA.

Dr. McGarrity questioned Dr. Moore's request for the RAC to postpone a recommendation on this issue. He said on both the RAC Working Group on Environmental Release and also the Working Group on Definitions, that for over two and one-half years, there had been very active participation of other federal agencies including USDA, EPA, and FDA.

Dr. Davis asked if there had been any request for a joint meeting between the RAC and the EPA BSAC, to which Mr. Mitchell replied that the BSAC as of this date still does not exist.

In reply to a question from Dr. Sharples as to why the RAC Working Group on Release into the Environment had not been asked to evaluate Dr. Gottesman's proposed amendment, Dr. Talbot explained that a working group is often called together when there is a proposal to develop, whereas in this case Dr. Gottesman had already developed the proposal and it was then published in the Federal Register for everyone to comment on it. Dr. Gottesman also noted that there is a great deal of overlap of membership between the Working Group on Release into the Environment and the Working Group on Definitions and that the latter did discuss the proposal. Dr. Sharples responded that a few key members of the Working Group on Release into the Environment had not been included in the Working Group on Definitions and she would like to see the Working Group on Release into the Environment called back to participate in this decision.

Dr. Sue Tolin of the USDA urged the RAC to consider the proposal as a scientific issue without reference to what other agencies are doing. Dr. Milewski of EPA agreed and said that RAC's function is to make recommendations to Dr. Wyngaarden who sits on the BSCC where coordination can and should occur.

Dr. Musgrave called the question and Mr. Mitchell reviewed the wording of the proposed amendment. Dr. Gottesman suggested that sections b and c of her proposal could be voted on separately and that Dr. Walters' suggestion of a footnote could be included as a "friendly amendment".

Dr. Cohen then asked for a point of information concerning work with deletion mutants in the laboratory without the intention of release into the environment. Dr. Gottesman and Dr. Talbot noted that these types of experiments were already exempt under the current Guidelines.

Dr. Pirone questioned the footnote proposed by Dr. Walters, and said it sounds like RAC is "ducking out on" rather than resolving the issue. Dr. Gottesman dropped the idea of including Dr. Walter's footnote in her motion.

There being no other discussion on the motion, Mr. Mitchell put the motion, in its original form, as duly moved and seconded to a vote. The motion was carried with a vote

of 16 in favor, 2 opposed and 2 abstentions.

Mr. Mitchell noted that an official photograph of the RAC would be taken. The retiring members of RAC who were in attendance were then presented Certificates of Appreciation signed by the Secretary of Health and Human Services for their service to the RAC, the National Institutes of Health, the Public Health Service, the Department of Health and Human Services and the nation. Those in attendance were Dr. McGonigle, Dr. Clowes and Dr. Gottesman. Those not in attendance, Dr. Mills and Dr. Joklik, were acknowledged as well and their certificates will be sent to them. Mr. Mitchell, in presenting the certificates, acknowledged the individual part each member had played and he thanked them for their many hours of hard work and the contributions that each had made to the reputation of the RAC. He stated that, despite retiring, the current members of RAC will continue to serve until such time as a replacement is appointed.

V. PROPOSAL TO ADD BACILLUS SPHAERICUS TO APPENDIX C-V

Dr. Clowes presented the proposal (tabs 1263 and 1269/II), which was a request by Dr. William Burke, Jr., Associate Professor of Microbiology at Arizona State University, that:

> "*Bacillus sphaericus* be added to the list of Gram positive bacteria described in Appendix C-V of the May 7, 1986 Guidelines which states that, 'Recombinant DNA molecules derived entirely from extrachromosomal elements of the organisms listed below (includding shuttle vectors constructed from vectors described in Appendix C), propagated and maintained in the organisms listed below are exempt from the Guidelines.'"

Dr. Clowes stated that Dr. Burke is working with *Bacillus sphaericus* of which there are a number of species that are pathogenic for mosquito larvae. Previously, in January, 1986, the RAC reviewed a recommendation from a working group considering a request from Dr. Richard Novick to extend the numbers of microorganisms exempted from the Guidelines based on the fact that they readily exchange genetic material. This resulted in addition to the Guidelines of Appendix C-V, entitled "Extrachromosomal Elements of Gram Positive Organisms." The list in Appendix C-V includes many *Bacillus* species.

Dr. Clowes stated that Dr. Burke would like to have *Bacillus sphaericus* added to Appendix C-V and Dr. Burke provided much positive evidence to show that this organism does have plasmids which can freely transfer to other organisms in Appendix C-V including *Bacillus subtilis* and *Bacillus licheniformis*. Dr. Burke has cited experiments in which he has transferred, using protoplast transformation, broad host-range plasmids from *Staphylococcus aureus* to *B. sphaericus* and has shown they are quite stable. Dr. Burke has also shown by co-cultivation he can transfer and maintain a broad host-range plasmid from *Streptomyces facaelis* into *B. sphaericus*.

Thus, Dr. Clowes stated, Dr. Burke has demonstrated the fact that *B. sphaericus* would be an appropriate addition to Appendix C-V and that he was fully in favor of such a recommendation.

Both Dr. Cohen and Dr. Davis agreed with Dr. Clowes that the presentation was thorough and well presented and neither could disagree in any way with Dr. Clowes' recommendation.

Mr. Mitchell then asked for anyone in opposition to such a proposal, and seeing no opposition called for Dr. Clowes to make a formal motion to add *Bacillus sphaericus*

to Appendix C-V of the Guidelines.

Dr. Clowes moved the addition of *Bacillus sphaericus* to become a part of Appendix C-V of the Guidelines and the motion was duly seconded by Dr. Cohen.

Mr. Mitchell called for discussion on the motion and hearing none put the motion to a vote. The result of the voting was an approval of the motion by a unanimous vote of 19 in favor, none opposed, and no abstentions.

VI. PROPOSED AMENDMENT OF SECTION III-A-4

Dr. Walters presented the proposed amendment (tabs 1261/I, 1265, 1270, 1271) as requested to be on the agenda by the Committee for Responsible Genetics (CRG) in a letter dated March 26, 1986 (tab 1265). This statement was duly published in the Federal Register of June 25, 1986 (tab 1261) and proposes text be added at the end of Section III-A-4 of the Guidelines as follows:

> "The RAC will not review and the NIH will not approve any human genetic therapy:
>
> "1. that is not aimed solely at the relief of a life-threatening or severely disabling condition; or
>
> "2. that could alter germ line cells.
>
> "Furthermore, the RAC will not review and the NIH will not approve any in vitro recombinant DNA experiments that alter human germ line cells or early human embryos."

Dr. Walters then briefly outlined the rationale presented with the proposal which was divided into four parts.

Somatic Cell Therapy for the Treatment of Disease

Dr. Walters said that here the authors argue that human trials of genetic therapy should await results of successful animal tests and that in the case of successful clinical treatment for some human disorders the research community will seek an expanded use of gene therapy beyond the initial range of cases where gene therapy has support of social consensus. And finally, the authors are urging RAC to establish in advance boundaries for "restricted zones of application of human somatic cell gene therapy."

Enhancement Therapies

Dr. Walters explained that the authors argue that use of somatic cell gene therapy to change such characteristics as height or skin tone would raise profound ethical problems.

Genetic Therapy for the Prevention of Disease

Dr. Walters presented the authors' view that it could be possible that employers may require an employee at some future time to undergo gene therapy for environmentally induced disease, rather than the employer removing the toxic disease-causing material from the workplace; limiting gene therapy to relief of life-threatening or severely disabling conditions would exclude such improper actions by employers.

Genetic Manipulation of the Human Germ Line

Dr. Walters pointed out the authors argue that genetic additions or deletions in the

sperm, egg, or zygote would be tantamount to experimentation on future generations and would also set a direct path to programs of eugenics.

Dr. Walters said the proposed amendment had been referred to the RAC Human Gene Therapy Subcommittee for consideration at its meeting of August 8, 1986. The results of the meeting can be found at tab 1271 entitled, Recommendation to RAC Regarding Proposal from Committee for Responsible Genetics. The subcommittee recommendation, explained Dr. Walters, was that the RAC not add new restrictions to Section III-A-4 of the Guidelines.

The Human Gene Therapy Subcommittee agreed that gene therapy should be attempted only for life-threatening or severely disabling conditions, but believed that this is already covered in the "Points to Consider in the Design and Submission of Human Somatic-Cell Gene Therapy Protocols." The entire thrust of Part I-A of the "Points to Consider," which deals with objectives and rationale of gene therapy protocols, is to ask about the seriousness of the disease and the availability of alternative therapies.

The subcommittee agreed that only somatic cell approaches to gene therapy should be considered at the present time. Indeed the title of the "Points to Consider" includes the phrase "somatic-cell gene therapy." However, the subcommittee was reluctant to speculate about what other approaches to gene therapy might become technically feasible in the future or to express a blanket disapproval of possible alternative approaches. Dr. Walters stated that the phrase, "At present," in paragraph 7 of the "Points to Consider" is meant to convey that current policy is not to entertain proposals for germ line therapy, but a willingness to consider new evidence if it emerges in the future.

Dr. Walters stated the subcommittee questioned the wording of the CRG's proposed Guideline change to exclude human genetic therapy "that could alter germ line cells," in that this would seem to rule out hypothetical unintended side effects on sperm or egg cells of a seriously ill patient, despite somatic-cell gene therapy being the only reasonable treatment for that patient. Dr. Walters underlined that unintended side effects on reproductive cells are currently accepted in cases where the patient consents to having toxic chemotherapy or radiation therapy directed at certain parts of the body and stated that the subcommittee felt it was unwarranted to set up a different standard for possible unintended side effects to apply to human somatic-cell gene therapy. Dr. Walters added that the "Points to Consider" document does ask investigators to specifically look for germ line effects in laboratory studies on animals (pg. 13 of Points to Consider).

Regarding in vitro experiments with sperm or egg cells, Dr. Walters noted that this concerns haploid cells only--separate sperm or egg cells--because if the two have gotten together and fertilization has occurred it falls under the next point concerning early human embryos. He pointed out that the subcommittee simply disagreed with the proposed exclusion of in vitro experiments that alter haploid human sperm or egg cells by means of recombinant DNA techniques. Such experiments are already covered in the Guidelines under Section III-C. The subcommittee was concerned that such a prohibition would impede potentially valuable research on haploid cells.

Dr. Walters then stated that the final type of experiment covered in the CRG's proposed Guideline change involves in vitro recombinant DNA experiments with early human embryos. Such experiments, if ever proposed, would be governed by Department of Health and Human Services regulations on human in vitro fertilization. Dr. Walters said that at the time of the August 8th subcommittee meeting, the members of the subcommittee believed that the Health Research Extension Act of 1985, P.L. 99-158, had placed a 3-year moratorium on research with human embryos. A closer reading of the statute suggests, however, that it applies only to implanted or formerly implanted

embryos and fetuses and not to pre-implantation embryos; therefore alternative wording to the second sentence of point 2 on page 2 of the subcommittee recommendations (tab 1271) has been worked out and should read as follows:

> "The subcommittee understands that human germ line cells would be covered by provisions for cells in tissue culture in the NIH Guidelines for Research Involving Recombinant DNA Molecules, and that HHS support for research involving human in vitro fertilization is precluded by regulation unless reviewed by an Ethical Advisory Board which must render advice as to the acceptability of the procedures."

Dr. Walters explained that this language is contained in tab 1278, which was distributed to the members of the RAC at today's meeting. He further noted that this language had been circulated on September 19 to all subcommittee members; comments were solicited from subcommittee members but no objections were received from any of them. Dr. Walters further stated that even though the subcommittee ultimately decided not to recommend a change in the Guidelines they believe that open discussion of these issues in a public forum, such as the RAC, is essential to the formulation of a sound public policy on human gene therapy. Dr. Walters added that at the appropriate moment he would move the recommendation of the Human Gene Therapy Subcommittee, as amended, be accepted by the RAC. Mr. Mitchell thanked Dr. Walters and called upon Dr. Epstein for his comments as a secondary reviewer.

Dr. Epstein stated that in general he concurred with the subcommittee's position. One of the major concerns he had with the CRG's requested changes was that in several places the language was so vague that it might lead into great difficulty in interpretation of what types of research should or should not be done. In particular he pointed to the terms "life-threatening" and "severely disabling," and said putting such words into the Guidelines would lead to interminable arguments as to what constitutes "life-threatening" or "severely disabling" conditions. He said there is no way of making a dichotomy between conditions that clearly will warrant therapy of this sort and conditions that clearly will not warrant therapy of this sort by the use of terms such as "life-threatening" or "severely disabling"; as times goes on, if these therapies prove successful, we may wish to change, on a case-by-case basis, the types of conditions that are treated. In the accompanying rationale from the CRG, the term "enhancement therapies" is used, apparently in an attempt to try to clearly discriminate these types of approaches from those which are "life-threatening" or "severely disabling", but if one thinks about the broad range of therapeutic maneuvers that are used medically, this kind of dichotomy is not clearly establishable and we today, in many ways, already use what would fall within the definition of "enhancement therapies" for legitimate medical needs.

Concerning the CRG's proposed addition to the Guidelines of the words "that could alter germline cells," Dr. Epstein said that "could" is a very broad and difficult word to deal with. Dr. Epstein said that there may be legitimate reasons for doing in vitro experiments on sperm or egg cells and that precluding such experimentation would not serve any useful goal and might inhibit possible work in the future that could be of tremendous benefit. Dr. Epstein said that there is an implicit assumption in the CRG proposal that any type of germ line therapy that one might envision in the future is on the face of it a bad thing, and yet there may be some serious genetic disorders where that may be a better approach than today's approaches involving prenatal diagnosis and abortion. Therefore, whereas he concurred with recommendations that at the present time there not be any attempts to alter the germ line or the genetic constitution of early embryos, he could not be sure that at some time in the future there might not be a clearly beneficial reason to do so.

Mr. Mitchell called on Nachama Wilker, Executive Director of the Committee for

Responsible Genetics. She read from a prepared statement which was distributed to the RAC which is attached to these minutes as Attachment II.

After Ms. Wilker's statement, Mr. Mitchell called on Dr. Stuart Newman. Dr. Newman stated he is a molecular embryologist and a member of the advisory board of the CRG. He said that somatic gene therapy seemed likely to present insurmountable technical problems in the short-run both with respect to achieving appropriate gene expression in differentiated cells and with respect to the very small number of diseases that can be cured by transplantation of somatic tissues, genetically engineered or otherwise.

However, he stated, genetic modification of early embryos at present is technically feasible and he cited modifications which extend to the germ line which have been accomplished in mice to produce double-sized mice and mice with an inherited defect of the Type I collagen gene. He said that if RAC rejects the CRG's proposal it is likely in the near future to receive proposals for human applications of germ line techniques which is both proven in animals and has much wider potential medical and commercial applicability than somatic techniques.

He said since even two parents with a dominant deleterious genetic defect such as Huntington's disease can still give rise to homozygous normal offspring, germ line therapy is not necesssary to ensure normal offspring of the genetically diseased parents. However, the more likely rationale, in Dr. Newman's opinion, for therapy on early embryos would be the introduction of traits not characteristic of either parent's genotype, for instance enhanced height.

Dr. Newman stated that, "Our experience is that any technique that is proven feasible, not specifically prohibited by regulation, and for which there is a commerical market will eventually be applied and sold." He further questioned how it is possible to judge whether human germ line therapy is safe when the consequences may not show up until subsequent generations. He further stated that, "disapproval of the CRG's motion will situate future deliberations on germ line therapy within the realm of the state of the technique and represent dubious progress towards turning the human species into an experimental system."

Mr. Mitchell called on Dr. Colin Gracey. Dr. Gracey stated he is a university chaplain and convenor of the Biogenetics Working Group of the Forum for Faith in the Future of the Episcopal Diocese of Massachusetts, and a member of the executive council of the Committee for Responsible Genetics. He stated that in submitting its proposed addition to the Guidelines, the CRG was seeking a clearer and more definitive statement as to how research and clinical uses of this important technology shall proceed.

Dr. Gracey said that, "there is widespread concern in our society that what can be done directs and determines what will be done. It is a concern that technical feasibility, rather than the counsel of human wisdom, becomes the measure for proceeding. The potential and promise of human gene therapy awakens this concern once again and public confidence on this matter will be influenced by the framing of public policy."

Dr. Gracey stated that the CRG proposal would provide substantive counsel on appropriate uses for proceeding with human gene therapy, ensuring that as it comes into practice that it does so with due caution and with sensitivity toward existing social consensus. He stated, an initial restriction to use in life-threatening or seriously disabling conditions would delineate the uses of gene therapy for which there appears a consensus to proceed. And if the CRG's proposal were accepted, it would necessitate changes in the Guidelines at some future time before extended use could be granted, but such future proposed changes would have the benefit of the experience with gene therapy experiments to date as well as provide adequate opportunity for public debate on any issues at hand.

Dr. Gracey stated that the CRG agreed with the position taken in the "Points to Consider" as regards proposals for germ line alterations, but believes that the "Points to Consider" is not as strong a policy statement as would be made by amending the Guidelines as proposed. He said that the CRG proposal uses the term "review" in the sense that the word "entertain" is used in the "Points to Consider", and that the intent of the proposal is not "to circumscribe the RAC's responsibility to remain open to developments in genetic technology and to review any and all material that comes to its attention."

Dr. Walters stated that the RAC Human Gene Therapy Subcommittee agreed with many of the concerns raised by the CRG. He believed that the first diseases anticipated to be proposed for human gene therapy will be precisely the types the CRG has described. The subcommittee is trying to anticipate an area of biomedical innovation. He gave the credit for this forward thinking to Mr. Mitchell who asked the RAC and the subcommittee to respond to the Presidential Commission Report Splicing Life which resulted in the RAC having a "Points to Consider" ready and waiting for the first proposals to perform somatic-cell gene therapy in human beings. The scientific community had been cooperating well and that there are no indications that any researchers in the United States are attempting to do anything other than the types of research envisioned in the "Points to Consider." Thus, there is a good public mechanism in place for review of somatic-cell gene therapy proposals; to move beyond the current mechanism at this time is unnecessary.

Dr. Epstein asked for clarification from the CRG as to its proposal, "that the RAC will not review and the NIH will not approve any in vitro recombinant DNA experiments that alter human germ line cells or early human embryos." He stated that in discussion at the RAC meeting this was referred to in terms of gene therapy with the implication that these cells would be used with fertilization techniques and reimplanted. Dr. Epstein stated there was a difference between such and experimentation on germ cells themselves which are never reimplanted. He asked for clarification of this point by the CRG.

Dr. Newman responded by stating that if the interest is in studying basic mechanisms then there are many animal models available for study with plenty of research to still be done. Dr. Newman stated, in contrast to work with animals, "that if the realm of research moves into working with human material then the agenda, either explicit or hidden, will be that the ultimate purpose is to modify human germ cells for the purpose of constructing better human beings... It seems to us that there are many good reasons to draw the line before doing modifications of human germ line cells because by incremental steps it will eventually lead to enhancement therapies in the germ line with unknown consequences to future generations."

Dr. Epstein then asked Dr. Newman if he felt that the only conceivable use for germ line therapy would be for enhancement therapies rather than treatment of otherwise untreatable genetic disorders. Dr. Newman responded that that in his opinion genetic disorders are validly treated in people who already exist and have genetic disease. If you are making genetic modifications to zygotes, you are "constructing an individual who doesn't yet exist." If the purpose was to ensure that families that have certain genetic defects would have normal children, "ordinary genetics would ensure that if appropriate selection were available." If that's not the goal, "then the goal must be something on the order of growth enhancement... If it is easier to enhance growth by genetic therapy on the zygote, people will demand it, and if not prohibited by statute or by recommendation there will be a market for it and it will be done." Dr. Epstein pointed to the fact that at present there are quite vocal people who believe that current methods of prenatal diagnosis and selective abortion are reprehensible.

Dr. Walters added that the Human Gene Therapy Subcommittee has attempted to keep abreast of laboratory science results that may be pertinent to human gene therapy or other kinds of human genetic alterations, with state-of-the-art lectures from experts in

the field, and that although transgenic laboratory studies are taking place in animals, there is no indication that any investigator is considering applying this technique to human beings.

Mr. Mitchell pointed out that at the January, 1986, meeting the RAC devoted the entire afternoon to three noted experts, Drs. Martin, Miller, and Parkman, who set forth the general science pertaining to this area of activity and that it was all directed towards severe genetic diseases. Further as relates to the public education concerning such experimentation, the NIH Recombinant DNA Technical Bulletin published a substantial portion of those remarks in the current issue in an effort to stimulate discussion and knowledge in this area.

Dr. Walters moved "That the RAC approve the recommendations of the Human Gene Therapy Subcommittee, as set forth in tab 1271, with the amendment given in tab 1278." The motion was seconded by Dr. Johnson .

Dr. Davis commended Ms. Wilker for what he perceived to be a major shift in position between the original CRG submission and Ms. Wilker's statement before the RAC. Dr. Davis said Ms. Wilker's use of the term "at this time" is a significant change from the original CRG position which he said he had found to be "ultra-conservative, somewhat resembling perhaps that of the more extreme fundamentalist kinds of religions which are so certain that they're right that there's no room for the kind of democratic process of pragmatic adjustment and shifts from time to time that we're acustomed to in our society." He said the issue now seemed to boil down to whether these issues should be dealt with in the Guidelines or simply as it is at present in the "Points to Consider" document.

Dr. Davis stated he agreed with Dr. Newman that it is now possible to instill genes into animal germ cells. However, since a very high percentage of the cells so treated fail and among those that are viable a certain percentage have grave defects introduced in them, no responsible medical researcher would want to undertake such experiments in humans at this time.

Ms. Wilker said that CRG has brought the issue up, so that public discussion can take place well in advance of the RAC receiving any such proposals, as opposed to discussing the matter while a proposal is on the table. Mr. Mitchell said that the RAC Human Gene Therapy Subcommittee meetings are announced well ahead of time and are always open to the public; hopefully CRG members could attend the meetings. Ms. Wilker stated that she appreciates that the process is an open one, but the meetings are still somewhat limited because of their taking place in the Washington, D.C. area; there may be persons who desire to comment who are in other parts of the country. She said, "it's not a comment, necessarily, that the process as it is right now is a problem, but that the process needs to be expanded, and I'm not necessarily putting that in the purview of the RAC itself."

Dr. Newman said "Dr. Davis said that the techniques would have to be very much better established in mice before it would be contemplated to do germ line genetic engineering on humans, and that seems very much to miss the point that we put forward, which is that no matter how well established in mice these techniques became, to do it on human beings would be to be making human beings and the human species as a whole, because of course we'd have the progeny of genetically engineered individuals, into an experimental system, and this is precisely what we oppose."

Dr. Miller from the FDA stated that he believed several assertions made by the CRG were ill-chosen or inaccurate. He stated that even if the technology were available to attempt germ line gene therapy, that the first attempts would very unlikely be aimed at enhancement or at attempted insertion in dominant genetic diseases, and rather would more likely be an attempt to intervene in recessive genetic diseases where there were

two affected parents, homozygous recessives, where the probability of producing affected offspring would be 100 percent.
Secondly, he believed it disingenuous to suggest there is widespread consensus, before the first human trials of a new technique. He pointed to the first clinical trials of the Jarvik artificial heart and oral contraceptives where the safety and efficacy were really unknown. He said the reason one does clinical trials is that the nuances of these techniques in man are not known, and cannot be known, before they are done, and that this is the reason for stringent regulation by local IRBs and central oversight by agencies such as the FDA.

Ms. Wilker responded that the reason the CRG is raising these issues at this point in time is that they see the science on the threshhold of new development and that they believe it is time for slow progress and for raising questions before moving ahead. She stated that with certain technologies that we use, such as low-dose radiation, we are still learning the risks and benefits, and that we should learn from our experience with these technologies and look closely at emerging technologies.

Dr. Korwek stated he was generally opposed to proposals which set out prohibitory language such as, "the RAC will not... and the NIH will not..." If the aim of the CRG is to encourage open discussion, it would be rather better to leave the status quo. Further, he felt ambiguity contained in some of the CRG proposed language further clouds the issues of what is to be prohibited. Therefore, he was opposed to the proposal.

Hearing no further comment, Mr. Mitchell called for a vote on the motion to accept the recommendations of the RAC Human Gene Therapy Subcommittee as set forth in tab 1271, and as amended by tab 1278. With a vote of 18 in favor, none opposed, and one abstention, the motion was carried.

Mr. Mitchell thanked the members of the Committee for Responsible Genetics and hoped that they attend future meetings of the RAC Subcommittee on Human Gene Therapy.

VII. PROPOSED AMENDED POINTS TO CONSIDER IN THE DESIGN AND SUBMISSION OF HUMAN SOMATIC-CELL GENE THERAPY PROTOCOLS

Mr. Mitchell called on Dr. Walters to discuss the proposed amended "Points to Consider in the Design and Submission of Human Somatic-Cell Gene Therapy Protocols" (tabs 1262, 1272, 1273, 1276).

Dr. Walters reminded the RAC that there had been a commitment made to review the "Points to Consider" at least annually for possible revision. He stated that tab 1272 specifies four changes which were proposed by the Subcommittee at its August 8, 1986, meeting and is followed by a draft of the document incorporating these changes. Dr. Walters went through each of the changes with the RAC. He stated that there had been distributed to the RAC additional technical amendments which are, in part, a response to comments of one of the subcommittee members who could not be at the August 8, 1986, meeting. These amendments are:

> "Page 3, footnote 1: Revise and add RNA. 'Section III-A-4 applies both to recombinant DNA and to DNA or RNA derived from recombinant DNA.'
>
> "Page 4, footnote 2: Update Federal Register reference. '...please see the Federal Register, Volume 51, p. 23311, 1986.'
>
> "Page 10, part (b): Revise list of contaminating materials. '...eliminate any contaminating materials (for example, VL30 RNA, other nucleic acids, or proteins) or...'

"Page 10, part (c): Revise new point, so that it does not ask investigators to demonstrate the absence of something. '(c) If co-cultivation is employed, what kinds of cells are being used for co-cultivation? What steps are being taken (and assays used with their sensitivity) to detect and eliminate any contaminating materials? Specifically, what tests are being done to assess the material to be returned to the patient for the presence of live or killed donor cells or other non-vector materials (for example, VL30 sequences) originating from those cells?'

"Page 10, part (d): Revise new point, so that it does not ask investigators to demonstrate absence. '(d) If methods other than those covered by a-c are used to introduce new genetic information into target cells, what steps are being taken to detect and eliminate any contaminating materials? What are...?'

"Page 12, part b, third line: Add a word for clarification. 'In what percentage of cells does expression from the added DNA occur?'"

Dr. Walters moved that the RAC accept the revised "Points to Consider" at tab 1272 with the further technical amendments just discussed, reflecting the recommendations of the Human Gene Therapy Subcommittee and its consultants. Dr. Epstein seconded the motion.

Dr. Epstein noted that tab 1276 contains a number of suggestions from Dr. Howard Temin, most of which were accepted, but one of which (page 6, line 1) was not. Dr. Temin had pointed out that it is possible that cells other than bone marrow cells might be used. Dr. Epstein stated Dr. Temin's suggestion could be accomplished by eliminating the words "bone marrow" from this sentence which currently reads, "...e.g., by inserting a properly functioning gene into a patient's bone marrow cells in vitro...."

Dr. Johnson and Dr. Pirone both noted that the language begins with "e.g." and is meant only as an example. Dr. Walters stated that if there were no qualifier on the phrase "into a patient's cells," it could be interpreted that this new gene could go into any cells including possibly germ line cells or reproductive cells, whichy is not the intention. Dr. Epstein agreed to drop this issue for now, but requested the next time the subcommittee meets, they consider changing this sentence to something like, "The purpose of somatic-cell gene therapy is to treat an individual patient's somatic cells," and then add on examples if appropriate.

Dr. Rapp asked the meaning of the term "contamination" in the new text which had been added as page 10, part (d); Dr. Epstein replied it presumably meant the same as in part (b) on the same page which had been previously defined. Dr. Neiman noted that in part (c) on the same page, the clarifying text, "(e.g., VL30 sequences)" appears.

The motion was put to a vote by the Chair and was passed unanimously, by a vote of 19 to zero with no abstentions.

Mr. Mitchell then called for any other business that any member desired to bring before the committee. Dr. Johnson asked about the committee appointed by the NIH Director to review the *Pseudorabies* vaccine field test and wondered if the panel had concluded their report. Dr. Talbot replied that the committee had not finished their

work but that it was anticipated to be concluded within the next few weeks.

VIII. FUTURE MEETING DATES

Mr. Mitchell directed the committee's attention to tab 12?3, a listing of future meeting dates, and reminded the members that the next two meetings of the RAC would take place on February 2, 1987 and June 15, 1987.

Mr. Mitchell noted that the Institute of Medicine is planning a symposium on human gene therapy in Washington, D.C. on October 15-16, 1986, and noted Dr. Walters is playing a key role in the program.

IX. ADJOURNMENT

Mr. Mitchell called for any other announcements or business to come before the committee, and hearing none asked for a motion to adjourn. The motion was made by Dr. Davis, seconded by Dr. Cohen, and after duly voting on the motion Mr. Mitchell declared the meeting adjourned.

_____

William J. Gartland, Jr., Ph.D.
Executive Secretary

I hereby certify that, to the best of my knowledge, the foregoing Minutes and Attachments are accurate and complete.

Date: _____

_____

Robert E. Mitchell, LL.B.
Chair
Recombinant DNA Advisory Committee

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

RECOMBINANT DNA ADVISORY COMMITTEE

BUILDING 1, WILSON HALL
BETHESDA, MARYLAND

FEBRUARY 2, 1987

AGENDA*

I.	CALL TO ORDER AND INTRODUCTION OF NEW MEMBERS			9:00 a.m.
II.	MINUTES OF SEPTEMBER 29, 1986, MEETING	#1288	Dr. Walters Dr. Bowman	9:05 a.m.
III.	REPORT OF THE WORKING GROUP ON DEFINITIONS AND PROPOSED REVISION OF SECTION III-A-2	#1285 1286/II 1289 A 1289 B 1289 C 1289 D 1289 H	Dr. McGarrity Dr. Gottesman Dr. Korwek Dr. Sharples Dr. Vidaver Dr. Clowes	9:15 a.m.
IV.	PROPOSED REVISION OF SECTION I-B OR SECTION III-A-2	#1285 1286/III 1287 1289 A 1289 C 1289 D 1289 E 1289 F 1289 H	Dr. McGarrity Dr. Gottesman Dr. Korwek Dr. Sharples Dr. Vidaver Dr. Clowes	10:30 a.m.
	LUNCH			12:30 p.m.
V.	PROPOSED AMENDMENTS OF SECTIONS I-A AND III-A	#1283 1286/I 1289 A 1289 B 1289 H	Dr. Johnson Dr. Korwek Dr. Davis	1:30 p.m.

*All times on this agenda are estimates. The actual time for consideration of an item may be earlier or later than indicated.

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VI. PROPOSED REVISIONS OF APPENDICES C-II, C-III, AND C-IV.....#1284.....Dr. McKinney.....3:00 p.m.
1286/IV Dr. McGarrity
1289 A Dr. Cohen
1289 B
1289 D
1289 F
1289 G

VII. REPORT FROM HUMAN GENE THERAPY SUBCOMMITTEE.....Dr. Walters.....4:30 p.m.

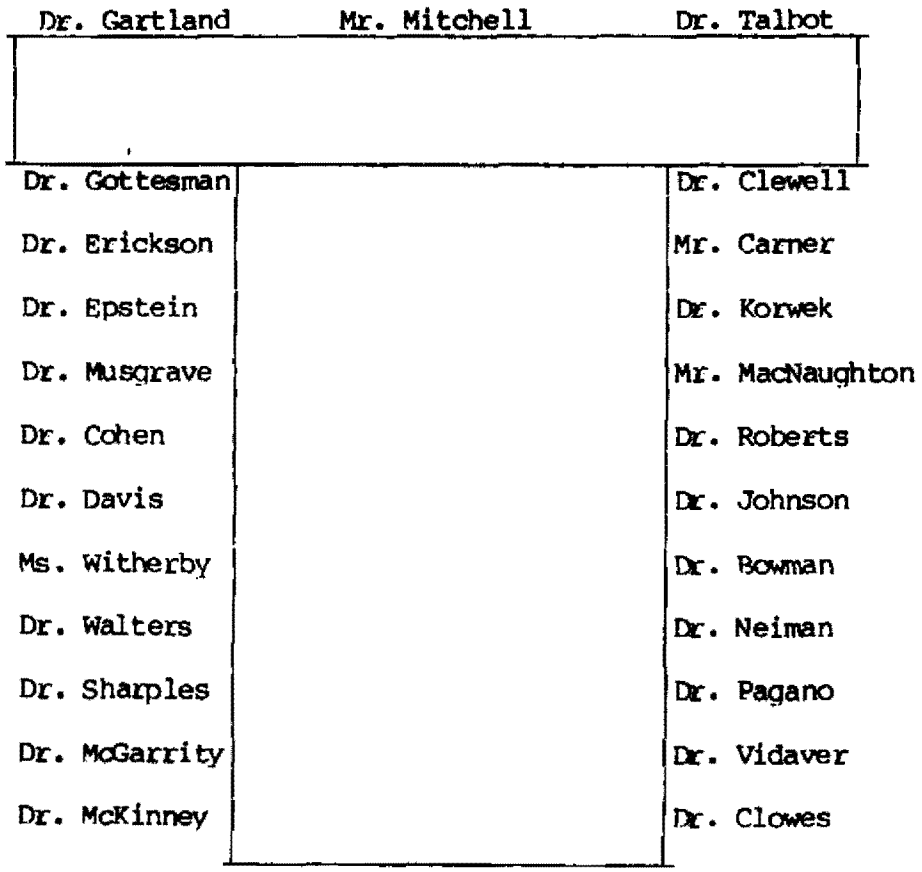
VIII. FUTURE MEETING DATES.....4:45 p.m.

IX. ADJOURNMENT.....5:00 p.m.

DEPARTMENT OF HEALTH AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH
RECOMBINANT DNA ADVISORY COMMITTEE

Building 1, Wilson Hall
Bethesda, Maryland

February 2, 1987



Materials
for
Meeting

D O O R

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federal register

Wednesday
May 7, 1986

Part III

Department of Health and Human Services

National Institutes of Health

Guidelines for Research Involving Recombinant DNA Molecules; Notice

ERRATUM

p.16969 - Appendix C-I, line 3,
insert word viral after eukaryotic

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Guidelines for Research Involving Recombinant DNA Molecules

May 1986.

These NIH Guidelines supersede earlier versions and will be in effect until further notice.

Table of Contents

I. Scope of the Guidelines

I–A—Purpose

The purpose of these Guidelines is to specify practices for constructing and handling (i) recombinant DNA molecules and (ii) organisms and viruses containing recombinant DNA molecules.

I–B—Definition of Recombinant DNA Molecules

In the context of these Guidelines, recombinant DNA molecules are defined as either (i) molecules which are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or (ii) DNA molecules that result from the replication of those described in (i) above.

Synthetic DNA segments likely to yield a potentially harmful polynucleotide or polypeptide (e.g., a toxin or a pharmocologically active agent) shall be considered as equivalent to their natural DNA counterpart. If the synthetic DNA segment is not expressed *in vivo* as a biologically active polynucleotide or polypeptide product, it is exempt from the Guidelines.

I–C—General Applicability

The Guidelines are applicable to all recombinant DNA research within the United States or its territories which is conducted at or sponsored by an institution that receives any support for recombinant DNA research from the National Institutes of Health (NIH). This includes research performed by NIH directly.

An individual receiving support for research involving recombinant DNA must be associated with or sponsored by an institution that can and does assume the responsibilities assigned in these Guidelines.

The Guidelines are also applicable to projects done abroad if they are supported by NIH funds. If the host country, however, has established rules for the conduct of recombinant DNA projects, then a certificate of compliance with those rules may be submitted to NIH in lieu of compliance with the NIH Guidelines. The NIH reserves the right to withhold funding if the safety practices to be employed abroad are not reasonably consistent with the NIH Guidelines.

I–D—General Definitions

The following terms, which are used throughout the Guidelines, are defined as follows:

I–D–1. "Institution" means any public or private entity (including Federal, State, and local government agencies).

I–D–2. "Institutional Biosafety Committee" or "IBC" means a committee that (i) meets the requirements for membership specified in Section IV–B–2, and (ii) reviews, approves, and oversees projects in accordance with the responsibilities defined in Sections IV–B–3 and IV–B–3.

I–D–3. "NIH Office of Recombinant DNA Activities" or "ORDA" means the office within NIH with responsibility for (i) reviewing and coordinating all activities of NIH related to the Guidelines, and (ii) performing other duties as defined in Section IV–C–3.

I–D–4. "Recombinant DNA Advisory Committee" or "RAC" means the public advisory committee that advises the Secretary, the Assistant Secretary for Health, and the Director, NIH, concerning recombinant DNA research. The RAC shall be constituted as specified in Section IV–C–2.

I–D–5. "Director, NIH" or "Director" means the Director, NIH, or any other officer or employee of NIH to whom authority has been delegated.

II. Containment

Effective biological safety programs have been operated in a variety of laboratories for many years. Considerable information, therefore, already exists for the design of physical containment facilities and the selection of laboratory procedures applicable to organisms carrying recombinant DNAs [3–16]. The existing programs rely upon mechanisms that, for convenience, can be divided into two categories: (i) A set of standard practices that are generally used in microbiological laboratories; and (ii) special procedures, equipment, and laboratory installations that provide physical barriers which are applied in varying degrees according to the estimated biohazard. Four biosafety levels (BL) are described in Appendix G. These biosafety levels consist of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities appropriate for the operations performed and the hazard posed by agents and for the laboratory function and activity. Biosafety level 4 (BL4) provides the most stringent containment conditions, BL1 the least stringent.

Experiments on recombinant DNAs by their very nature lend themselves to a third containment mechanism—namely, the application of highly specific biological barriers. In fact, natural barriers do exist which limit either (i) the infectivity of a *vector* or *vehicle* (plasmid or virus) for specific hosts, or (ii) its dissemination and survival in the environment. The vectors that provide the means for replication of the recombinant DNAs and/or the host cells in which they replicate can be genetically designed to decrease by many orders of magnitude the probability of dissemination of recombinant DNAs outside the laboratory. Further details on biological containment may be found in Appendix I.

As these three means of containment are complementary, different levels of containment appropriate for experiments with different recombinants can be established by applying various combinations of the physical and biological barriers along with a constant use of the standard practices. We consider these categories of containment separately in order that such combinations can be conveniently expressed in the Guidelines.

In constructing these Guidelines, it was necessary to define boundary conditions for the different levels of physical and biological containment and for the classes of experiments to which they apply. We recognize that these definitions do not take into account all existing and anticipated information on special procedures that will allow particular experiments to be carried out under different conditions than indicated here without affecting risk. Indeed, we urge that individual investigators devise simple and more effective containment procedures, and that investigators and IBCs recommend changes in the Guidelines to permit their use.

III. Guidelines for Covered Experiments

Part III discusses experiments involving recombinant DNA. These experiments have been divided into four classes:

III–A. Experiments which require specific RAC review and NIH and IBC approval before initiation of the experiment;

III–B. Experiments which require IBC approval before initiation of the experiment;

III–C. Experiments which require IBC notification at the time of initiation of the experiment;

III–D. Experiments which are exempt from the procedures of the Guidelines.

IF AN EXPERIMENT FALLS INTO BOTH CLASS III–A AND ONE OF THE OTHER CLASSES, THE RULES PERTAINING TO CLASS III–A MUST BE FOLLOWED. If an experiment falls into Class III–D and into either Class III–B or III–C as well, it can be considered

exempt from the requirements of the Guidelines.

Changes in containment levels from those specified here may not be instituted without the express approval of the Director, NIH [see Sections IV–C–1–b–(1), IV–C–1–b–(2), and subsections].

III–A—Experiments That Require RAC Review and NIH and IBC Approval Before Initiation

Experiments in this category cannot be initiated without submission of relevant information on the proposed experiment to NIH, the publication of the proposal in the **Federal Register** for thirty days of comment, review by the RAC, and specific approval by NIH. The containment conditions for such experiments will be recommended by RAC and set by NIH at the time of approval. Such experiments also require the approval of the IBC before initiation. *Specific experiments already approved* in this section and the appropriate containment conditions are listed in Appendices D and F. If an experiment is similar to those listed in Appendices D and F, ORDA may determine appropriate containment conditions according to case precedents under Section IV–C–1–b–(3)–(g).

If the experiments in this category are submitted for review to another Federal agency, the submitter shall notify ORDA; ORDA may then determine that such review serves the same purpose, and based on that determination, notify the submitter that no RAC review will take place, no NIH approval is necessary, and the experiment may proceed upon approval from the other Federal agency.

III–A–1. Deliberate formation of recombinant DNAs containing genes for the biosynthesis of toxic molecules lethal for vertebrates at an LD_{50} of less than 100 nanograms per kilogram body weight (e.g., microbial toxins such as the botulinum toxins, tetanus toxin, diphtheria toxin, *Shigella dysenteriae* neurotoxin). Specific approval has been given for the cloning in *E. coli* K–12 of DNAs containing genes coding for the biosynthesis of toxic molecules which are lethal to vertebrates at 100 nanograms to 100 micrograms per kilogram body weight. Containment levels for these experiments are specified in Appendix F.

III–A–2. Deliberate release into the environment of any organism containing recombinant DNA, except certain plants as described in Appendix L.

III–A–3. Deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire it naturally [2], if such acquisition could compromise the use of the drug to control disease agents in human or veterinary medicine or agriculture.

III–A–4. Deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into human subjects [21]. The requirement for RAC review should not be considered to preempt any other required review of experiments with human subjects. Institutional Review Board (IRB) review of the proposal should be completed before submissin to NIH.

III–B—Experiments That Require IBC Approval Before Initiation

Investigators performing experiments in this category must submit to their IBC, prior to initiation of the experiments, a registration document that contains a description of: (i) The source(s) of DNA; (ii) the nature of the inserted DNA sequences; (iii) the hosts and vectors to be used; (iv) whether a deliberate attempt will be made to obtain expression of a foreign gene, and, if so, what protein will be produced; and (v) the containment conditions specified in these Guidelines. This registration document must be dated and signed by the investigator and filed only with the local IBC. The IBC shall review all such proposals prior to initiation of the experiments. Requests for lowering of containment for experiments in this category will be considered by NIH [see Section IV–C–1–b–(3)].

III–B–1—Experiments Using Human or Animal Pathogens (Class 2, Class 3, Class 4, or Class 5 Agents [1]) as Host-Vector Systems

III–B–1–a. Experiments involving the introduction of recombinant DNA into Class 2 agents can be carried out at BL2 containment.

III–B–1–b. Experiments involving the introduction of recombinant DNA into Class 3 agents can be carried out at BL3 containment.

III–B–1–c. Experiments involving the introduction of recombinant DNA into Class 4 agents can be carried out at BL4 containment.

III–B–1–d. Containment conditions for experiments involving the introduction of recombinant DNA into Class 5 agents will be set on a case-by-case basis following ORDA review. A U.S. Department of Agriculture (USDA) permit is required for work with Class 5 agents [18, 20].

III–B–2—Experiments in Which DNA From Human or Animal Pathogens (Class 2, Class 3, Class 4, or Class 5 Agents [1]) is Cloned in Nonpathogenic Prokaryotic or Lower Eukaryotic Host-Vector Systems

III–B–2–a. Recombinant DNA experiments in which DNA from Class 2 or Class 3 agents [1] is transferred into nonpathogenic prokaryotes or lower eukaryotes may be performed under BL2 containment. Recombinant DNA experiments in which DNA from Class 4 agents is transferred into nonpathogenic prokaryotes or lower eukaryotes can be performed at BL2 containment after demonstration that only a totally and irreversibly defective fraction of the agent's genome is present in a given recombinant. In the absence of such a demonstration, BL4 containment should be used. Specific lowering of containment of BL1 for particular experiments can be approved by the IBC. Many experiments in this category will be exempt from the Guidelines (see Sections III–D–4 and III–D–5). Experiments involving the formation of recombinant DNAs for certain genes coding for molecules toxic for vertebrates require RAC review and NIH approval (see Section III–A–1) or must be carried out under NIH specified conditions as described in Appendix F.

III–B–2–b. Containment conditions for experiments in which DNA from Class 5 agents is transferred into nonpathogenic prokaryotes or lower eukaryotes will be determined by ORDA following a case-by-case review. A USDA permit is required for work with Class 5 agents [18, 20].

III–B–3—Experiments Involving the Use of Infectious Animal or Plant DNA or RNA Viruses or Defective Animal or Plant DNA or RNA Viruses in the Presence of Helper Virus in Tissue Culture Systems

Caution: Special care should be used in the evaluation of containment levels for experiments which are likely to either enhance the pathogenicity (e.g., insertion of a host oncogene) or to extend the host range (e.g., introduction of novel control elements) of viral vectors under conditions which permit a productive infection. In such cases, serious consideration should be given to raising the physical containment by at least one level.

Note.—Recombinant DNA molecules or RNA molecules derived therefrom, which contain less than two-thirds of the genome of any eukaryotic virus (all virus from a single Family [17] being considered identical [19]), may be considered defective and can be used

in the absence of helper under the conditions specified in Section III–C.

III–B–3–a. Experiments involving the use of infectious Class 2 animal viruses [1] or defective Class 2 animal viruses in the presence of helper virus can be performed at BL2 containment.

III–B–3–b. Experiments involving the use of infectious Class 3 animal viruses [1] or defective Class 3 animal viruses in the presence of helper virus can be carried out at BL3 containment.

III–B–3–c. Experiments involving the use of infectious Class 4 viruses [1] or defective Class 4 viruses in the presence of helper virus may be carried out under BL4 containment.

III–B–3–d. Experiments involving the use of infectious Class 5 [1] viruses or defective Class 5 viruses in the presence of helper virus will be determined on a case-by-case basis following ORDA review. A USDA permit is required for work with Class 5 pathogens [18, 20].

III–B–3–e. Experiments involving the use of infectious animal or plant viruses or defective animal or plant viruses in the presence of helper virus not covered by Sections III–B–3–a, III–B–3–b, III–B–3–c, or III–B–3–d may be carried out under BL1 containment.

III–B–4—Recombinant DNA Experiments Involving Whole Animals or Plants

III–B–4–a. Recombinant DNA, or RNA molecules derived therefrom, from any source except for greater than two-thirds of a eukaryotic viral genome may be transferred to any non-human vertebrate organism and propagated under conditions of physical containment comparable to BL1 and appropriate to the organism under study [2]. It is important that the investigator demonstrate that the fraction of the viral genome being utilized does not lead to productive infection. A USDA permit is required for work with Class 5 agents [18, 20].

III–B–4–b. For all experiments involving whole animals and plants and not covered by Section III–B–4–a, the appropriate containment will be determined by the IBC [22].

III–B–5—Experiments Involving More Than 10 Liters of Culture

The appropriate containment will be decided by the IBC. Where appropriate, Appendix K, *Physical Containment for Large-Scale Uses of Organisms Containing Recombinant DNA Molecules*, should be used.

III–C. Experiments That Require IBC Notice Simultaneously With Initiation of Experiments

Experiments not included in Sections III–A, III–B, III–D, and subsections of these sections are to be considered in Section III–C. All such experiments can be carried out at BL1 containment. For experiments in this category, a registration document as described in Section III–B must be dated and signed by the investigator and filed with the local IBC at the time of initiation of the experiment. The IBC shall review all such proposals, but IBC review prior to initiation of the experiment is not required. (The reader should refer to the policy statement in the first two paragraphs of Section IV–A.)

For example, experiments in which all components derive from non-pathogenic prokaryotes and non-pathogenic lower eukaryotes fall under Section III–C and can be carried out at BL1 containment.

CAUTION: Experiments Involving Formation of Recombinant DNA Molecules Containing no more than Two-Thirds of the Genome of any Eukaryotic Virus. Recombinant DNA molecules containing no more than two-thirds of the genome of any eukaryotic virus (all viruses from a single Family [17] being considered identical [19]) may be propagated and maintained in cells in tissue culture using BL1 containment. For such experiments, it must be shown that the cells lack helper virus for the specific Families of defective viruses being used. If helper virus is present, procedures specified under Section III–B–3 should be used. The DNA may contain fragments of the genome of viruses from more than one Family but each fragment must be less than two-thirds of a genome.

III–D—Exempt Experiments

The following recombinant DNA molecules are exempt from these Guidelines and no registration with the IBC is necessary:

III–D–1. Those that are not in organisms or viruses.

III–D–2. Those that consist entirely of DNA segments from a single nonchromosomal or viral DNA source though one or more of the segments may be a synthetic equivalent.

III–D–3. Those that consist entirely of DNA from a prokaryotic host including its indigenous plasmids or viruses when propagated only in that host (or a closely related strain of the same species) or when transferred to another host by well established physiological means; also, those that consist entirely of DNA from an eukaryotic host including its chloroplasts, mitochondria, or plasmids (but excluding viruses) when propagated only in that host (or a closely related strain of the same species).

III–D–4. Certain specified recombinant DNA molecules that consist entirely of DNA segments from different species that exchange DNA by known physiological processes though one or more of the segments may be a synthetic equivalent. A list of such exchangers will be prepared and periodically revised by the Director, NIH, with advice of the RAC after appropriate notice and opportunity for public comment (see Section IV–C–1–b–(1)–(c)). Certain classes are exempt as of publication of these revised Guidelines. This list is in Appendix A. An updated list may be obtained from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 3B10, Bethesda, Maryland 20892.

III–D–5. Other classes of recombinant DNA molecules—if the Director, NIH, with advice of the RAC, after appropriate notice and opportunity for public comment, finds that they do not present a significant risk to health or the environment (see Section IV–C–1–b–(1)–(c)). Certain classes are exempt as of publication of these revised Guidelines. The list is in Appendix C. An updated list may be obtained from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 3B10, Bethesda, Maryland 20892.

IV. Roles and Responsibilities

IV–A—Policy

Safety in activities involving recombinant DNA depends on the individual conducting them. The Guidelines cannot anticipate every possible situation. Motivation and good judgment are the key essentials to protection of health and the environment.

The Guidelines are intended to help the institution, Institutional Biosafety Committee (IBC), Biological Safety Officer (BSO), and Principal Investigator (PI) determine the safeguards that should be implemented. These Guidelines will never be complete or final, since all conceivable experiments involving recombinant DNA cannot be foreseen. Therefore, *it is the responsibility of the institution and those associated with it to adhere to the intent of the Guidelines as well as to their specifics.*

Each institution (and the IBC acting on its behalf) is responsible for ensuring that recombinant DNA activities comply with the Guidelines. General recognition of institutional authority and

responsibility properly establishes accountability for safe conduct of the research at the local level.

The following roles and responsibilities constitute an administrative framework in which safety is an essential and integral part of research involving recombinant DNA molecules. Further clarifications and interpretations of roles and responsibilities will be issued by NIH as necessary.

IV–B—Responsibility of the Institution

IV–B–1. General Information. Each institution conducting or sponsoring recombinant DNA research covered by these Guidelines is responsible for ensuring that the research is carried out in full conformity with the provisions of the Guidelines. In order to fulfill this responsibility, the institution shall:

IV–B–1–a. Establish and implement policies that provide for the safe conduct of recombinant DNA research and that ensure compliance with the Guidelines. The institution as part of its general responsibilities for implementing the Guidelines may establish additional procedures as deemed necessary to govern the institution and its components in the discharge of its responsibilities under the Guidelines. This may include: (i) Statements formulated by the institution for general implementation of the Guidelines, and (ii) whatever additional precautionary steps the institution may deem appropriate.

IV–B–1–b. Establish an IBC that meets the requirements set forth in Section IV–B–2 and carries out the functions detailed in Section IV–B–3.

IV–B–1–c. If the institution is engaged in recombinant DNA research at the BL3 or BL4 containment level, appoint a BSO, who shall be a member of the IBC and carry out the duties specified in Section IV–B–4.

IV–B–1–d. Require that investigators responsible for research covered by these Guidelines comply with the provisions of Section IV–B–5 and assist investigators to do so.

IV–B–1–e. Ensure appropriate training for the IBC chairperson and members, the BSO, PIs, and laboratory staff regarding the Guidelines, their implementation, and laboratory safety. Responsibility for training IBC members may be carried out through the IBC chairperson. Responsibility for training laboratory staff may be carried out through the PI. The institution is responsible for seeing that the PI has sufficient training but may delegate this responsibility to the IBC.

IV–B–1–f. Determine the necessity in connection with each project for health surveillance of recombinant DNA research personnel, and conduct, if found appropriate, a health surveillance program for the project. [The "Laboratory Safety Monograph" (LSM) discusses various possible components of such a program—for example, records of agents handled, active investigation of relevant illnesses, and the maintenance of serial serum samples for monitoring serologic changes that may result from the employees' work experience. Certain medical conditions may place a laboratory worker at increased risk in any endeavor where infectious agents are handled. Examples given in the LSM include gastrointestinal disorders and treatment with steroids, immunosuppressive drugs, or antibiotics. Workers with such disorders or treatment should be evaluated to determine whether they should be engaged in research with potentially hazardous organisms during their treatment or illness. Copies of the LSM are available from ORDA.]

IV–B–1–g. Report within 30 days to ORDA any significant problems with and violations of the Guildelines and significant research-related accidents and illnesses, unless the institution determines that the PI or IBC has done so.

IV–B–2. Membership and Procedures of the IBC. The institution shall establish an IBC whose responsibilities need not be restricted to recombinant DNA. The committee shall meet the following requirements:

IV–B–2–a. The IBC shall comprise no fewer than five members so selected that they collectively have experience and expertise in recombinant DNA technology and the capability to assess the safety of recombinant DNA research experiments and any potential risk to public health or the environment. At least two members shall not be affiliated with the institution (apart from their membership on the IBC) and shall represent the interest of the surrounding community with respect to health and protection of the environment. Members meet this requirement if, for example, they are officials of State or local public health or environmental protection agencies, members of other local governmental bodies, or persons active in medical, occupational health, or environmental concerns in the community. The BSO, mandatory when research is being conducted at the BL3 and BL4 levels, shall be a member (see Section IV–B–4).

IV–B–2–b. In order to ensure the competence necessary to review recombinant DNA activities, it is recommended that: (i) The IBC include persons with expertise in recombinant DNA technology, biological safety, and physical containment; (ii) the IBC include, or have available as consultants, persons knowledgeable in institutional commitments and policies, applicable law, standards of professional conduct and practice, community attitudes, and the environment; and (iii) at least one member be from the laboratory technical staff.

IV–B–2–c. The institution shall identify the committee members by name in a report to ORDA and shall include relevant background information on each member in such form and at such times as ORDA may require.

IV–B–2–d. No member of an IBC may be involved (except to provide information requested by the IBC) in the review or approval of a project in which he or she has been or expects to be engaged or has a direct financial interest.

IV–B–2–e. The institution, who is ultimately responsible for the effectiveness of the IBC, may establish procedures that the IBC will follow in its initial and continuing review of applications, proposals, and activities. (IBC review procedures are specified in Section IV–B–3–a.)

IV–B–2–f. Institutions are encouraged to open IBC meetings to public whenever possible, consistent with protection of privacy and proprietary interests.

IV–B–2–g. Upon request, the institution shall make available to the public all minutes of IBC meetings and any documents submitted to or received from funding agencies which the latter are required to make available to the public. If comments are made by members of the public on IBC actions, the institution shall forward to NIH both the comments and the ICB's response.

IV–B–3. Functions of the IBC. On behalf of the institution, the IBC is responsible for:

IV–B–3–a. Reviewing for compliance with the NIH Guidelines recombinant DNA research as specified in Part III conducted at or sponsored by the institution, and approving those research projects that it finds are in conformity with the Guidelines. This review shall include:

IV–B–3–a–(1). An independent assessment of the containment levels required by these Guidelines for the proposed research, and

IV–B–3–a–(2). An assessment of the facilities, procedures, and practices, and of the training and expertise of recombinant DNA personnel.

IV–B–3–b. Notifying the PI of the results of their review.

IV–B–3–c. Lowering containment levels for certain experiments as specified in Sections III–B–2.

IV–B–3–d. Setting containment levels as specified in Section III–B–4–b and III–B–5.

IV–B–3–e. Reviewing periodically recombinant DNA research being conducted at the institution to ensure that the requirements of the Guidelines are being fulfilled.

IV–B–3–f. Adopting emergency plans covering accidental spills and personnel contamination resulting from such research.

Note.—Basic elements in developing specific procedures for dealing with major spills of potentially hazardous materials in the laboratory are detailed in the LSM. Included are information and references on decontamination and emergency plans. The NIH and the Centers for Disease Control are available to provide consultation and direct assistance, if necessary, as posted in the LSM. The institution shall cooperate with the State and local public health departments reporting any significant research-related illness or accident that appears to be a hazard to the public health.

IV–B–3–g. Reporting within 30 days to the appropriate institutional official and to ORDA any significant problems with or violations of the Guidelines and any significant research-related accidents or illnesses unless the IBC determines that the PI has done so.

IV–B–3–h. The IBC may not authorize initiation of experiments not explicitly covered by the Guidelines until NIH (with the advice of the RAC when required) establishes the containment requirement.

IV–B–3–i. Performing such other functions as may be delegated to the IBC under Section IV–B–1.

IV–B–4. Biological Safety Officer. The institution shall appoint a BSO if it engages in recombinant DNA research at the BL3 or BL4 containment level. The officer shall be a member of the IBC, and his or her duties shall include (but need not be limited to):

IV–B–4–a. Ensuring through periodic inspections that laboratory standards are rigorously followed;

IV–B–4–b. Reporting to the IBC and the institution all significant problems with and violations of the Guidelines and all significant research-related accidents and illnesses of which the BSO becomes aware unless the BSO determines that the PI has done so;

IV–B–4–c. Developing emergency plans for dealing with accidental spills and personnel contamination and investigating recombinant DNA research laboratory accidents.

IV–B–4–d. Providing advice on laboratory security;

IV–B–4–e. Providing technical advice to the PI and the IBC on research safety procedures.

Note.—See the LSM for additional information on the duties of the BSO.

IV–B–5. Principal Investigator (PI). On behalf of the institution, the PI is responsible for complying fully with the Guidelines in conducting any recombinant DNA research.

IV–B–5. PI—General. As part of this general responsibility, the PI shall:

IV–B–5–a–(1). Initiate or modify no recombinant DNA research requiring approval by the IBC prior to initiation (see Sections III–A and III–B) until that research or the proposed modification thereof has been approved by the IBC and has met all other requirements of the Guidelines;

IV–B–5–a–(2). Determine whether experiments are covered by Section III–C and follow the appropriate procedures;

IV–B–5–a–(3). Report within 30 days to the IBC and NIH (ORDA) all significant problems with and violations of the Guidelines and all significant research-related accidents and illnesses;

IV–B–5–a–(4). Report to the IBC and to NIH (ORDA) new information bearing on the Guidelines;

IV–B–5–a–(5). Be adequately trained in good microbiological techniques;

IV–B–5–a–(6). Adhere to IBC-approved emergency plans for dealing with accidental spills and personnel contamination; and

IV–B–5–a–(7). Comply with shipping requirements for recombinant DNA molecules. (See Appendix H for shipping requirements and the LSM for technical recommendations.)

IV–B–5–b. Submissions by the PI to NIH. The PI shall:

IV–B–5–b–(1). Submit information to NIH (ORDA) in order to have new host-vector systems certified;

IV–B–5–b–(2). Petition NIH with notice to the IBC for exemptions to these Guidelines;

IV–B–5–b–(3). Petition NIH with concurrence of the IBC for approval to conduct experiments specified in Section III–A of the Guidelines;

IV–B–5–b–(4). Petition NIH for determination of containment for experiments requiring case-by-case review;

IV–B–5–b–(5). Petition NIH for determination of containment for experiments not covered by the Guidelines.

IV–B–5–c. Submissions by the PI to the IBC. The PI shall:

IV–B–5–c–(1). Make the initial determination of the required levels of physical and biological containment in accordance with the Guidelines;

IV–B–5–c–(2). Select appropriate microbiological practices and laboratory techniques to be used in the research;

IV–B–5–c–(3). Submit the initial research protocol if covered under Guidelines Section III–A, III–B, or III–C (and also subsequent changes—e.g., changes in the source of DNA or host-vector system) to the IBC for review and approval or disapproval; and

IV–B–5–c–(4). Remain in communication with the IBC throughout the conduct of the project.

IV–B–5–d. PI Responsibilities Prior to Initiating Research. The PI is responsible for:

IV–B–5–d–(1). Making available to the laboratory staff copies of the protocols that describe the potential biohazards and the precautions to be taken;

IV–B–5–d–(2). Instructing and training staff in the practices and techniques required to ensure safety and in the procedures for dealing with accidents; and

IV–B–5–d–(3). Informing the staff of the reasons and provisions for any precautionary medical practices advised or requested, such as vaccinations or serum collection.

IV–B–5–e. PI Responsibilities During the Conduct of the Research. The PI is responsible for:

IV–B–5–e–(1). Supervising the safety performance of the staff to ensure that the required safety practices and techniques are employed;

IV–B–5–e–(2). Investigating and reporting in writing to ORDA, the BSO (where applicable), and the IBC any significant problems pertaining to the operation and implementation of containment practices and procedures;

IV–B–5–e–(3). Correcting work errors and conditions that may result in the release of recombinant DNA materials;

IV–B–5–e–(4). Ensuring the integrity of the physical containment (e.g., biological safety cabinets) and the biological containment (e.g., purity and genotypic and phenotypic characteristics).

IV–C—Responsibilities of NIH

IV–C–1. Director. The Director, NIH, is responsible for (i) establishing the NIH Guidelines for Research Involving Recombinant DNA Molecules, (ii) overseeing their implementation, and (iii) their final interpretation.

The Director has responsibilities under the Guidelines that involve ORDA and RAC. The ORDA's responsibilities under the Guidelines are administrative. Advice from the RAC is primarily

scientific and technical. In certain circumstances, there is specific opportunity for public comment with published response before final action.

IV–C–1–a. General Responsibilities of the Director, NIH. The responsibilities of the director shall include the following:

IV–C–1–a–(1). Promulgating requirements as necessary to implement the Guidelines;

IV–C–1–a–(2). Establishing and maintaining the RAC to carry out the responsibilities set forth in Section IV–C–2. The RAC's membership is specified in its charter and in Section IV–C–2;

IV–C–1–a–(3). Establishing and maintaining ORDA to carry out the responsibilities defined in Section IV–C–3.

IV–C–1–b. Specific Responsibilities of the Director, NIH. In carrying out the responsibilities set forth in this section, the director or a designee shall weigh each proposed action through appropriate analysis and consultation to determine that it complies with the Guidelines and presents no significant risk to health or the environment.

IV–C–1–b–(1). Major Actions. To execute major actions the director must seek the advice of the RAC and provide an opportunity for public and Federal agency comment. Specifically, the agenda of the RAC meeting citing the major actions will be published in the **Federal Register** at least 30 days before the meeting, and the director will also publish the proposed actions in the **Federal Register** for comment as least 30 days before the meeting. In addition, the director's proposed decision, at his discretion, may be published in the **Federal Register** for 30 days of comment before final action is taken. The director's final decision, along with response to the comments, will be published in the **Federal Register** and the *Recombinant DNA Technical Bulletin.* The RAC and IBC chairpersons will be notified of this decision:

IV–C–1–b–(1)–(a). Changing containment levels for types of experiments that are specified in the Guidelines when a major action is involved;

IV–C–1–b–(1)–(b). Assigning containment levels for types of experiments that are not explicitly considered in the Guidelines when a major action is involved;

IV–C–1–b–(1)–(c). Promulgating and amending a list of classes of recombinant DNA molecules to be exempt from these Guidelines because they consist entirely of DNA segments from species that exchange DNA by known physiological processes or otherwise do not present a significant risk to health or the environment;

IV–C–1–b–(1)–(d). Permitting experiments specified by Section III–A of the Guidelines;

IV–C–1–b–(1)–(e). Certifying new host-vector systems with the exception of minor modifications of already certified systems (the standards and procedures for certification are described in Appendix I–II–A. Minor modifications constitute, for example, those of minimal or no consequence to the properties relevant to containment); and

IV–C–1–b–(1)–(f). Adopting other changes in the Guidelines.

IV–C–1–b–(2). Lesser Actions. To execute lesser actions, the director must seek the advice of the RAC. The director's decision will be transmitted to the RAC and IBC chairpersons and publiched in the *Recombinant DNA Technical Bulletin:*

IV–C–1–b–(2)–(a). Interpreting and determining containment levels upon request by ORDA;

IV–C–1–b–(2)–(b). Changing containment levels for experiments that are specified in the Guidelines (see Section III);

IV–C–1–b–(2)–(c). Assigning containment levels for experiments not explicitly considered in the Guidelines;

IV–C–1–b–(2)–(d). Revising the "Classification of Etiologic Agents" for the purpose of these Guidelines [1].

IV–C–1–b–(3). Other Actions. The director's decision will be transmittede to the RAC and IBC chairpersons and published in the *Recombinant DNA Technical Bulletin:*

IV–C–1–b–(3)–(a). Interpreting the Guidelines for experiments to which the Guidelines specifically assign containment levels;

IV–C–1–b–(3)–(b). Setting containment under Section III–B–1–d and Section III–B–3–d;

IV–C–1–b–(3)–(c). Approving minor modifications of already certified host-vector systems (the standards and procedures for such modifications are described in Appendix I–II);

IV–C–1–b–(3)–(d). Decertifying already certified host-vector systems;

IV–C–1–b–(3)–(e). Adding new entries to the list of molecules toxic for vertebrates (see Appendix F);

IV–C–1–b–(3)–(f). Approving the cloning of toxin genes in host-vector systems other than *E. coli* K–12 (see Appendix F); and

IV–C–1–b–(3)–(g). Determining appropriate containment conditions for experiments according to case precedents developed under Section IV–C–1–b–(2)–(c).

IV–C–1–b–(4). The director shall conduct, support, and assist training programs in laboratory safety for IBC members, BSOs, PIs, and laboratory staff.

IV–C–2. Recombinant DNA Advisory Committee. The Recombinant DNA Advisory Committee (RAC) is responsible for carrying out specified functions cited below as well as others assigned under its charter or by the Secretary, HHS, the Assistant Secretary for Health, and the Director, NIH.

The committee shall consist of 25 members including the chair, appointed by the Secretary or his or her designee, at least fourteen of whom shall be selected from authorities knowledgeable in the fields of molecular biology or recombinant DNA research or in scientific fields other than molecular biology or recombinant DNA research, and at least six of whom shall be persons knowledgeable in applicable law, standards of professional conduct and practice, public attitudes, the environment, public health, occupational health, or related fields. Representatives from Federal agencies shall serve as non-voting members. Nominations for the RAC may be submitted to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 3B10, Bethesda, MD 20892.

All meetings of the RAC will be announced in the **Federal Register**, including tentative agenda items, 30 days in advance of the meeting with final agendas (if modified) available at least 72 hours before the meeting. No item defined as a major action under Section IV–C–1–b–(1) may be added to an agenda after it appears in the **Federal Register**.

The RAC shall be responsible for advising the Director, NIH, on the actions listed in Section IV–C–1–b–(1) and IV–C–1–b–(2).

IV–C–3. The Office of Recombinant DNA Activities. The ORDA shall serve as a focal point for information on recombinant DNA activities and provide advice to all within and outside NIH including Institutions, BSOs, PIs, Federal agencies, State and local governments and institutions in the private sector. The ORDA shall carry out such other functions as may be delegated to it by the Director, NIH, including those authorities described in Section IV–C–1–b–(3). In addition, ORDA shall be responsible for the following:

IV–C–3–a. Reviewing and approving IBC membership;

IV–C–3–b. Publishing in the **Federal Register**:

IV–C–3–b–(1). Announcements of RAC meetings and agendas at least 30 days in advance;

Note.—If the agenda for an RAC meeting is modified, ORDA shall make the revised agenda available to anyone upon request at least 72 hours in advance of the meeting.

IV–C–3–b–(2). Proposed major actions of the type falling under Section IV–C–1–b–(1) at least 30 days prior to the RAC meeting at which they will be considered; and

IV–C–3–b–(3). The NIH director's final decision on recommendations made by the RAC.

IV–C–3–c. Publishing the *Recombinant DNA Technical Bulletin;* and

IV–C–3–d. Serving as executive secretary of the RAC.

IV–C–4. Other NIH Components. Other NIH components shall be responsible for certifying maximum containment (BL4) facilities, inspecting them periodically, and inspecting other recombinant DNA facilities as deemed necessary.

IV–D—Compliance

As a condition for NIH funding of recombinant DNA research, institutions must ensure that such research conducted at or sponsored by the institution, irrespective of the source of funding, shall comply with these Guidelines. The policies on noncompliance are as follows:

IV–D–1. All NIH-funded projects involving recombinant DNA techniques must comply with the NIH Guidelines. Noncompliance may result in (i) suspension, limitation, or termination of financial assistance for such projects and of NIH funds for other recombinant DNA research at the institution, or (ii) a requirement for prior NIH approval of any or all recombinant DNA projects at the Institution.

IV–D–2. All non-NIH funded projects involving recombinant DNA techniques conducted at or sponsored by an institution that receives NIH funds for projects involving such techniques must comply with the NIH Guidelines. Noncompliance may result in: (i) Suspension, limitation, or termination of NIH funds for recombinant DNA research at the institution, or (ii) a requirement for prior NIH approval of any or all recombinant DNA projects at the institution.

IV–D–3. Information concerning noncompliance with the Guidelines may be brought forward by any person. It should be delivered to both NIH (ORDA) and the relevant Institution. The institution, generally through the IBC, shall take appropriate action. The institution shall forward a complete report of the incident to ORDA, recommending any further action.

IV–D–4. In cases where NIH proposes to suspend, limit, or terminate financial assistance because of noncompliance with the Guidelines, applicable DHHS and Public Health Service procedures shall govern.

IV–D–5. Voluntary Compliance. Any individual, institution, or institution that is not otherwise covered by the Guidelines is encouraged to conduct recombinant DNA research activities in accordance with the Guidelines through the procedures set forth in Part VI.

V. Footnotes and References of Sections I–IV

1. The original references to organisms as Class 1, 2, 3, 4 are replaced by the classification in the publication *Classification of Etiologic Agents on the Basis of Hazard*, 4th Edition, July 1974, U.S. Department of Health, Education, and Welfare, Public Health Service, Centers for Disease Control, Office of Biosafety, Atlanta, Georgia 30333.

The Director, NIH, with advice of the Recombinant DNA Advisory Committee, may revise the classification for the purposes of these Guidelines (see Section IV–C–1–b–(2)–(d)]. The revised list of organisms in each class is reprinted in Appendix B to these Guidelines.

2. In Part III of the Guidelines, there are a number of places where judgments are to be made. In all these cases the principal investigator is to make the judgment on these matters as part of his responsibility to "make the initial determination of the required levels of physical and biological containment in accordance with the Guidelines" (Section IV–B–5–c–(1)). In the cases falling under Sections III–A–2, or IV–C, this judgment is to be reviewed and approved by the IBC as part of its responsibility to make "an independent assessment of the containment levels required by these Guidelines for the proposed research" (Section IV–B–3–a–(1)]. If the IBC wishes, any specific cases may be referred to ORDA as part of ORDA's functions to "provide advice to all within and outside NIH" (Section IV–C–3), and ORDA may request advice from the RAC as part of the RAC's responsibility for "reviewing and determining containment levels upon request by ORDA" (Section IV–C–3–b–(2)–(a)).

3. *Laboratory Safety at the Center for Disease Control* (Sept. 1974). U.S. Department of Health, Education and Welfare Publication No. CDC 75–8118.

4. *Classification of Etiologic Agents on the Basis of Hazard* (4th Edition, July 1974). U.S. Department of Health, Education and Welfare. Public Health Service. Centers for Disease Control, Office of Biosafety, Atlanta, Georgia 30333.

5. *National Cancer Institute Safety Standards for Research Involving Oncogenic Viruses* (Oct. 1974). U.S. Department of Health, Education and Welfare Publication No. (NIH) 75–790.

6. *National Institutes of Health Biohazards Safety Guide* (1974). U.S. Department of Health, Education and Welfare, Public Health Service, National Institutes of Health. U.S. Government Printing Office, Stock No. 1740–00363.

7. *Biohazards in Biological Research* (1973). A. Hellman, M.N. Oxman, and R. Pollack (ed.) Cold Spring Harbor Laboratory.

8. *Handbook of Laboratory Safety* (1971). 2nd Edition, N.V. Steere (ed.). The Chemical Rubber Co., Cleveland.

9. Bodily, H.L. (1970). *General Administration of the Laboratory*, H.L. Bodily, E.L. Updyke, and J.O. Mason (eds.), Diagnostic Procedures for Bacterial, Mycotic and Parasitic Infections. American Public Health Association, New York, pp. 11–28.

10. Darlow, H.M. (1969). *Safety in the Microbiological Laboratory.* In J.R. Norris and D.W. Robbins (ed.), Methods in Microbiology. Academic Press, Inc., New York, pp. 169–204.

11. *The Prevention of Laboratory Acquired Infection* (1974). C.H. Collins, E.G. Hartley, and E. Hilsworth. Public Health Laboratory Service, Monograph Series No. 6.

12. Chatigny, M.A. (1961). *Protection Against Infection in the Microbiological Laboratory: Devices and Procedures.* In W.W. Umbreit (ed.): Advances in Applied Microbiology. Academic Press, New York, N.Y. 3:131–192.

13. *Design Criteria for Viral Oncology Research Facilities* (1975). U.S. Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, DHEW Publication No. (NIH) 75–891.

14. Kuehne, R.W. (1973). *Biological Containment Facility for Studying Infectious Disease.* Appl. Microbiol. 26–259–263.

15. Runkle, R.S., and G.B. Phillips (1969). *Microbial Containment Control Facilities.* Van Nostrand Reinhold, New York.

16. Chatigny, M.A., and D.I. Clinger (1969). *Contamination Control in Aerobiology.* In R.L. Dimmick and A.B. Akers (eds.). An Introduction to Experimental Aerobiology. John Wiley & Sons, New York, pp. 194–203.

17. As classified in the Third Report of the International Committee on Taxonomy of Viruses: Classification and Nomenclature of Viruses, R.E.F. Matthews, Ed. Intervirology 12 (129–296) 1979.

18. A USDA permit, required for import and interstate transport of pathogens, may be obtained from the Animal and Plant Health Inspection Service, USDA, Federal Building, Hyattsville, MD 20782.

19. I.e., the total of all genomes within a Family shall not exceed two-thirds of the genome.

20. All activities, including storage of variola and whitepox, are restricted to the single national facility (World Health Organization (WHO) Collaborating Center for Smallpox Research, Centers for Disease Control, in Atlanta).

21. Section III–A–4 covers only those experiments in which the intent is to modify stably the genome of cells of a human subject. Other experiments involving recombinant DNA in human subjects such as feeding of bacteria containing recombinant DNA or the administration of vaccines containing recombinant DNA are not covered in Section III–A–4 of the Guidelines.

22. For recombinant DNA experiments in which the intent is to modify stably the genome of cells of a human subject, see Section III–A–4.

VI. Voluntary Compliance

VI–A.—Basic Policy

Individuals, corporations, and institutions not otherwise covered by the Guidelines are encouraged to do so by following the standards and procedures set forth in Parts I–IV of the Guidelines. In order to simplify discussion, references hereafter to "institutions" are intended to encompass corporations, and individuals who have no organizational affiliation. For purposes of complying with the Guidelines, an individual intending to carry out research involving recombinant DNA is encouraged to affiliate with an institution that has an IBC approved under the Guidelines.

Since commercial organizations have special concerns, such as protection of proprietary data, some modifications and explanations of the procedures in Parts I–IV are provided below, in order to address these concerns.

VI–B—IBC Approval

The ORDA will review the membership of an institution's IBC, and where it finds the IBC meets the requirements set forth in Section IV–B–2 will give its approval to the IBC membership.

It should be emphasized that employment of an IBC member solely for purposes of membership on the IBC does not itself make the member an institutionally affiliated member for purposes of Section IV–B–2–a.

Except for the unaffiliated members, a member of an IBC for an institution not otherwise covered by the Guidelines may participate in the review and approval of a project in which the member has a direct financial interest so long as the member has not been, and does not expect to be, engaged in the project. Section IV–B–2–d is modified to that extent for purposes of these institutions.

VI–C—Certification of Host-Vector Systems

A host-vector system may be proposed for certification by the Director, NIH, in accordance with the procedures set forth in Appendix I–II–A.

In order to ensure protection for proprietary data, any public notice regarding a host-vector system which is designated by the institution as proprietary under Section VI–E–1 will be issued only after consultation with the institution as to the content of the notice.

VI–D—Requests for Exemptions and Approvals

Requests for exemptions or other approvals required by the Guidelines should be requested by following the procedures set forth in the appropriate sections in Parts I–IV of the Guidelines.

In order to ensure protection for proprietary data, any public notice regarding a request for an exemption or other approval which is designated by the institution as proprietary under Section VI–E–1 will be issued only after consultation with the institution as to the content of the notice.

VI–E—Protection of Proprietary Data

In general, the Freedom of Information Act requires Federal agencies to make their records available to the public upon request. However, this requirement does not apply to, among other things, "trade secrets and commercial and financial information obtained from a person and privileged or confidential." 18 U.S.C. 1905, in turn makes it a crime for an officer or employee of the United States or any Federal department or agency to publish, divulge, disclose, or make known "in any manner or to any extent not authorized by law any information coming to him in the course of his employment or official duties or by reason of any examination or investigation made by, or return, report or record made to or filed with, such department or agency or officer or employee thereof, which information concerns or relates to the trade secrets, [or] processes . . . of any person, firm, partnership, corporation, or association." This provision applies to all employees of the Federal Government, including special Government employees. Members of the Recombinant DNA Advisory Committee are "special Government employees."

VI–E–1. In submitting to NIH for purposes of complying voluntarily with the Guidelines, an institution may designate those items of information which the institution believes constitute trade secrets, privileged, confidential commercial, or financial information.

VI–E–2. If NIH receives a request under the Freedom of Information Act for information so designated, NIH will promptly contact the institution to secure its views as to whether the information (or some portion) should be released.

VI–E–3. If the NIH decides to release this information (or some portion) in response to a Freedom of Information request or otherwise, the institution will be advised; and the actual release will not be made until the expiration of 15 days after the institution is so advised except to the extent that earlier release in the judgment of the Director, NIH, is necessary to protect against an imminent hazard to the public or the environment.

VI–E–4. Presubmission Review.

VI–E–4–a. Any institution not otherwise covered by the Guidelines, which is considering submission of data or information voluntarily to NIH, may request presubmission review of the records involved to determine whether if the records are submitted NIH will or will not make part or all of the records available upon request under the Freedom of Information Act.

VI–E–4–b. A request for presubmission review should be submitted to ORDA along with the records involved. These records must be clearly marked as being the property of the institution on loan to NIH solely for the purpose of making a determination under the Freedom of Information Act. The ORDA will then seek a determination from the HHS Freedom of Information Officer, the responsible official under HHS regulations (45 CFR Part 5) as to whether the records involved (or some portion) are or are not available to members of the Public under the Freedom of Information Act. Pending such a determination the records will be kept separate from ORDA files, will be considered records of the institution and not ORDA, and will not be received as part of ORDA files. No copies will be made of the records.

VI–E–4–c. The ORDA will inform the institution of the HHS Freedom of Information Officer's determination and follow the institution's instructions as to whether some or all of the records involved are to be returned to the institution or to become a part of ORDA files. If the institution instructs ORDA to return the records, no copies or summaries of the records will be made or retained by HHS, NIH, or ORDA.

VI–E–4–d. The HHS Freedom of Information Officer's determination will represent that official's judgement at the time of the determination as to whether the records involved (or some portion) would be exempt from disclosure under the Freedom of Information Act if at the time of the determination the records were in ORDA files at a request were received for them under the Act.

Appendix A—Exemptions Under Section III–D–4

Section III–D–4 states that exempt from these Guidelines are "certain specified recombinant DNA molecules that consist entirely of DNA segments from different species that exchange

DNA by known physiological processes though one or more of the segments may be a synthetic equivalent. A list of such exchangers will be prepared and periodically revised by the Director, NIH, with advice of the RAC after appropriate notice and opportunity for public comment (see Section IV-C-1-b-(1)-(c)). Certain classes are exempt as of publication of these revised Guidelines. The list is in Appendix A."

Under Section III-D-4 of these Guidelines are recombinant DNA molecules that are: (1) Composed entirely of DNA segments from one or more of the organisms within a sublist and (2) to be propagated in any of the organisms within a sublist. (Classification of *Bergey's Manual of Determinative Bacteriology*, 8th edition. R. E. Buchanan and N. E. Gibbons, editors. Williams and Wilkins Company: Baltimore, 1974.)

Although these experiments are exempt, it is recommended that they be performed at the appropriate biosafety level for the host or recombinant organism (for biosafety levels see *Biosafety in Microbiological and Biomedical Laboratories*, 1st Edition (March 1984), U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, Atlanta, Georgia 30333, and National Institutes of Health, Bethesda, Maryland 20892).

Sublist A

1. Genus *Escherichia*
2. Genus *Shigella*
3. Genus *Salmonella* (including *Arizona*)
4. Genus *Enterobacter*
5. Genus *Citrobacter* (including *Levinea*)
6. Genus *Klebsiella*
7. Genus *Erwinia*
8. *Pseudomonas aeruginosa, Pseudomonas Putida* and *Pseudomonas fluorescens*
9. *Serratia marcescens*
10. *Yersinia enterocolitica*

Sublist B

1. *Bacillus subtilis*
2. *Bacillus licheniformis*
3. *Bacillus pumilus*
4. *Bacillus globigii*
5. *Bacillus niger*
6. *Bacillus natto*
7. *Bacillus amyloliquefaciens*
8. *Bacillus aterrimus*

Sublist C

1. *Streptomyces aureofaciens*
2. *Streptomyces rimosus*
3. *Streptomyces coelicolor*

Sublist D

1. *Streptomyces griseus*
2. *Streptomyces cyaneus*
3. *Streptomyces venezuelae*

Sublist E

1. One way hybrids of *Streptococcus mutans* or *Streptococcus lactis* DNA into *Streptococcus sanguis*.

Sublist F

1. *Streptococcus sanguis*
2. *Streptococcus pneumoniae*
3. *Streptococcus faecalis*
4. *Streptococcus pyogenes*
5. *Streptococcus mutans*

APPENDIX B—CLASIFICATION OF MICROORGANISMS ON THE BASIS OF HAZARD

Appendix B-I—Classification of Etiologic Agents

The original reference for this classification was the publication *Classification of Etiological Agents on the Basis of Hazard*, 4th edition, July 1974, U.S. Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, Office of Biosafety, Atlanta, Georgia 30333. For the purposes of these Guidelines, this list has been revised by the NIH [1].

Appendix B-I-A. Class 1 Agents. All bacterial, parasitic, fungal, viral, rickettsial, and chlamydial agents not included in higher classes.

Appendix B-I-B. Class 2 Agents.

Appendix B-I-B-1. Bacterial Agents.

Acinetobacter calcoaceticus
Actinobacillus-all species
Aeromonas hydrophila
Arizona hinshawii-all serotypes
Bacillus anthracis
Bordetella-all species
Borrelia recurrentis, B. vincenti
Campylobacter fetus
Campylobacter jejuni
Chlamydia psittaci
Chlamydia trachomatis
Clostridium botulinum, Cl. chauvoei, Cl. haemolyticum, Cl. histolyticum, Cl. novyi, Cl. septicum, Cl. tetani
Corynebacterium diphtheriae, C. equi, C. haemolyticum, C. pseudotuberculosis, C. pyogenes, C. renale
Edwardsiella tarda
Erysipelothrix insidiosa
Escherichia coli-all enteropathogenic, enterotoxigenic, enteroinvasive and strains bearing K1 antigen
Haemophilus ducreyi, H. influenzae
Klebsiella-all species and all serotypes
Legionella pneumophila
Leptospira interrogans-all serotypes
Listeria-all species
Moraxella-all species
Mycobacteria-all species except those listed in Class 3
Mycoplasma-all species except *Mycoplasma mycoides* and *Mycoplasma agalactiae*, which are in Class 3
Neisseria gonorrhoeae, N. meningitidis
Pasteurella-all species except those listed in Class 3
Salmonella-all species and all serotypes
Shigella-all species and all serotypes
Sphaerophorus necrophorus
Staphylococcus aureus
Streptobacillus moniliformis
Streptococcus pneumoniae
Streptococcus pyogenes
Treponema carateum, T. pallidum, and T. pertenue
Vibrio cholerae
Vibrio parahemolyticus
Yersinia enterocolitica

Appendix B-I-B-2. Fungal Agents.

Actinomycetes (including *Nocardia* species, *Actinomyces* species, and *Arachnia propionica*) [2]
Blastomyces dermatitidis
Cryptococcus neoformans
Paracoccidioides braziliensis

Appendix B-I-B-3. Parasitic Agents.

Endamoeba histolytica
Leishmania sp.
Naegleria gruberi
Schistosoma mansoni
Toxoplasma gondii
Toxocara canis
Trichinella spiralis
Trypanosoma cruzi

Appendix B-I-B-4. Viral, Rickettsial, and Chlamydial Agents.

Adenoviruses—human—all types
Cache Valley virus
Coxsackie A and B viruses
Cytomegaloviruses
Echoviruses—all types
Encephalomyocarditis virus (EMC)
Flanders virus
Hart Park virus
Hepatitus—associated antigen material
Herpes viruses-except *Herpesvirus simiae (Monkey B virus)* which is in Class 4
Corona viruses
Influenza viruses—all types except A/PR8/34, which is in Class 1
Langat virus
Lymphogranuloma venereum agent
Measles virus
Mumps virus
Parainfluenza virus—all types except Parainfluenza virus 3, SF4 strain, which is in Class 1
Polioviruses—all types, wild and attenuated
Poxviruses—all types except *Alastrim, Smallpox*, and *Whitepox* which are Class 5 and *Monkey pox* which depending on experiments is in Class 3 or Class 4
Rabies virus—all strains except *Rabies street virus* which should be classified in Class 3
Reoviruses—all types
Respiratory syncytial virus
Rhinoviruses—all types
Rubella virus
Simian viruses—all types except *Herpesvirus simiae (Monkey B virus)* and *Marburg virus* which are in Class 4
Sindbis virus
Tensaw virus
Turlock virus
Vaccinia virus
Varicella virus
Vesicular stomatitis virus [3]

Vole rickettsia
Yellow fever virus, 17D vaccine strain

Appendix B–I–C. Class 3 Agents.

Appendix B–I–C–1. Bacterial Agents.

Bartonella—all species
Brucella—all species
Francisella tularensis
Mycobacterium avium, M. bovis, M. tuberculosis
Pasteurella multocide type *B* ("buffalo" and other foreign virulent strains) [3]
Pseudomonas mallei [3]
Pseudomonas pseudomallei [3]
Yersinia pestis

Appendix B–I–C–2. Fungal Agents.

Coccidioides immitis
Histoplasma capsulatum
Histoplasma capsulatum var. *duboisii*

Appendix B–I–C–3. Parasitic Agents.

None.

Appendix B–I–C–4. Viral, Rickettsial, and Chlamydial Agents.

Monkey pox, when used *in vitro* [4]
Arboviruses-all strains except those in Class 2 and 4 (*Arboviruses* indigenous to the United States are in Class 3 except those listed in Class 2. *West Nile* and *Semliki Forest* viruses may be classified up or down depending on the conditions of use and geographical location of the laboratory.]
Dengue virus, when used for transmission or animal inoculation experiments
Lymphocytic choriomeningitis virus (LCM)
Rickettsia—all species except *Vole rickettsia* when used for transmission or animal inoculation experiments
Yellow fever virus—wild, when used *in vitro*

Appendix B–I–D. Class 4 Agents.

Appendix B–I–D–1. Bacterial Agents.

None.

Appendix B–I–D–2. Fungal Agents.

None.

Appendix B–I–D–3. Parasitic Agents.

None.

Appendix B–I–D–4. Viral, Rickettsial, and Chlamydial Agents.

Ebola fever virus
Monkey pox, when used for transmission or animal inoculation experiments [4]
Hemorrhagic fever agents, including *Crimean hemorrhagic fever, (Congo), Junin*, and *Machupo* viruses, and others as yet undefined
Herpesvirus simiae (Monkey B virus)
Lassa virus
Marburg virus
Tick-borne encephalitis virus complex, including *Russian spring-summer encephalitis, Kyasanur forest disease, Omsk hemorrhagic fever*, and *Central European encephalitis viruses*
Venezuelan equine encephalitis virus. epidemic strains, when used for transmission or animal inoculation experiments
Yellow fever virus—wild, when used for transmission or animal inoculation experiments

Appendix B–II—Classification of Oncogenic Viruses on the Basis of Potential Hazard [5]

Appendix B–II–A. Low-Risk Oncogenic Viruses.

Rous sarcoma
SV–40
CELO
Ad7–SV40
Polyoma
Bovine papilloma
Rat mammary tumor
Avian leukosis
Murine leukemia
Murine sarcoma
Mouse mammary tumor
Rat leukemia
Hamster leukemia
Bovine leukemia
Dog sarcoma
Mason-Pfizer monkey virus
Marek's
Guinea pig herpes
Lucke (Frog)
Adenovirus
Shope fibroma
Shope papilloma

Appendix B–II–B. Moderate-Risk Oncogenic Viruses.

Ad2–SV40
FeLV
HV Saimiri
EBV
SSV–1
GaLV
HV ateles
Yaba
FeSV

Appendix B–III—Class 5 Agents

Appendix B–III–A. Animal Disease Organisms Which are Forbidden Entry into the United States by Law.

Foot and mouth disease virus.

Appendix B–III–B. Animal Disease Organisms and Vectors Which are Forbidden Entry into the United States by USDA Policy.

African horse sickness virus
African swine fever virus
Besnoitia besnoiti
Borna disease virus
Bovine infectious petechial fever
Camel pox virus
Ephemeral fever virus
Fowl plague virus
Goat pox virus
Hog cholera virus
Louping ill virus
Lumpy skin disease virus
Nairobi sheep disease virus
Newcastle disease virus (Asiatic strains)
Mycoplasma mycoides (contagious bovine pleuropneumonia)
Mycoplasma agalactiae (contagious agalactia of sheep)
Rickettsia ruminatium (heart water)
Rift valley fever virus
Rhinderpest virus
Sheep pox virus
Swine vesicular disease virus
Teschen disease virus
Trypanosoma vivax (Nagana)
Trypanosoma evansi
Theileria parva (East Coast fever)
Theileria annulata
Theileria lawrencei
Theileria bovis
Theileria hirci
Vesicular exanthema virus
Wesselsbron disease virus
Zyonema

Appendix B–III–C. Organisms Which may not be Studied in the United States Except at Specified Facilities.

Small pox [4]
Alastrim [4]
White pox [4]

Appendix B–IV—Footnotes and References of Appendix B.

1. The original reference for this classification was the publication *Classification of Etiologic Agents on the Basis of Hazard*, 4th edition, July 1974, U.S. Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, Office of Biosafety, Atlanta, Georgia 30333. For the purposes of these Guidelines, this list has been revised by the NIH.

2. Since the publication of the classification in 1974 [1], the *Actinomycetes* have been reclassified as bacterial rather than fungal agents.

3. A USDA permit, required for import and interstate transport of pathogens, may be obtained from the Animal and Plant Health Inspection Service, USDA, Federal Building, Hyattsville, MD 20782.

4. All activities, including storage of variola and whitepox, are restricted to the single national facility [World Health Organization (WHO) Collaborating Center for Smallpox Research, Centers for Disease Control, in Atlanta].

5. *National Cancer Institute Safety Standards for Research Involving Oncogenic Viruses* (October 1974). U.S. Department of Health, Education, and Welfare Publication No. (NIH) 75–790.

6. U.S. Department of Agriculture, Animal and Plant Health Inspection Service.

Appendix C—Exemptions Under Section III–D–5

Section III–D–5 states that exempt from these Guidelines are "Other classes of recombinant DNA molecules if the Director, NIH, with advice of the RAC, after appropriate notice and opportunity for public comment finds that they do not present a significant risk to health or the environment (see Section IV–C–1–b–(1)–(c)). Certain classes are exempt as of publication of these revised Guidelines."

The following classes of experiments are exempt under Section III–D–5 of the Guidelines:

Appendix C–I—Recombinant DNAs in Tissue Culture.

Recombinant DNA molecules containing less than one-half of any eukaryotic genome [all viruses from a single Family (4) being considered identical (5)] that are propagated and maintained in cells in tissue culture are exempt from these Guidelines with the exceptions listed below.

Exceptions. Experiments described in Section III–A which require specific RAC review and NIH approval before initiation of the experiment.

Experiments involving DNA from Class 3, 4, or 5 organisms [1] or cells known to be infected with these agents.

Experiments involving the deliberate introduction of genes coding for the biosynthesis of molecules toxic for vertebrates (see Appendix F).

Appendix C–II—Experiments Involving E. coli K–12 Host-Vector Systems

Experiments which use *E. coli* K–12 host-vector systems, with the exception of those experiments listed below, are exempt from these Guidelines provided that: (i) the *E. coli* host shall not contain conjugation proficient plasmids or generalized transducing phages; and (ii) lambda or lambdoid or Ff bacteriophages or nonconjugative plasmids [2] shall be used as vectors. However, experiments involving the insertion into *E. coli* K–12 of DNA from prokaryotes that exchange genetic information [3] with *E. coli* may be performed with any *E. coli* K–12 vector (e.g., conjugative plasmid). When a nonconjugative vector is used, the *E. coli* K–12 host may contain conjugation-proficient plasmids either autonomous or integrated, or generalized transducing phages.

For these exempt laboratory experiments, BL1 physical containment conditions are recommended.

For large-scale (LS) fermentation experiments BL1–LS physical containment conditions are recommended. However, following review by the IBC of appropriate data for a particular host-vector system, some latitude in the application of BL1–LS requirements as outlined in Appendix K–II–A through K–II–F is permitted.

Exceptions. Experiments described in Section III–A which require specific RAC review and NIH approval before initiation of the experiment.

Experiments involving DNA from Class 3, 4, or 5 organisms [1] or from cells known to be infected with these agents may be conducted under containment conditions specified in Section III–B–2 with prior IBC review and approval.

Large-scale experiments (e.g., more than 10 liters of culture) require prior IBC review and approval (see Section III–B–5).

Experiments involving the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates (see Appendix F).

Appendix C–III—Experiments Involving Saccharomyces Host-Vector Systems

Experiments which use *Saccharomyces cerevisiae* host-vector systems, with the exception of experiments listed below, are exempt from these Guidelines.

Experiments which use *Saccharomyces uvarum* host-vector systems, with the exception of experiments listed below, are exempt from these Guidelines.

For these exempt laboratory experiments, BL1 physical containment conditions are recommended.

For large-scale fermentation experiments BL1–LS physical containment conditions are recommended. However, following review by the IBC of appropriate data for a particular host-vector system some latitude in the application of BL1–LS requirements as outlined in Appendix K–II–A through K–II–F is permitted.

Exceptions. Experiments described in Section III–A which require specific RAC review and NIH approval before initiation of the experiment.

Experiments involving Class 3, 4, or 5 organisms [1] or cells knowns to be infected with these agents may be conducted under containment conditions specified in Section III–B–2 with prior IBC review and approval.

Large-scale experiments (e.g., more than 10 liters of culture) require prior IBC review and approval (see Section III–B–5).

Experiments involving the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates (see Appendix F).

Appendix C–IV—Experiments Involving Bacillus subtilis Host-Vector Systems

Any asporogenic *Bacillus subtilis* strain which does not revert to a sporeformer with a frequency greater than 10^{-7} can be used for cloning DNA with the exception of those experiments listed below.

For these exempt laboratory experiments, BL1 physical containment conditions are recommended.

For large-scale fermentation experiments BL1–LS physical containment conditions are recommended. However, following review by the IBC of appropriate data for a particular host-vector system, some latitude in the application of BL1–LS requirements as outlined in Appendix K–II–A through K–II–F is permitted.

Exceptions. Experiments described in Section III–A which require specific RAC review and approval before initiation of the experiment.

Experiments involving Class 3, 4, or 5 organisms [1] or cells known to be infected with these agents may be conducted under containment conditions specified in Section III–B–2 with prior IBC review and approval.

Large-scale experiments (e.g., more than 10 liters of culture) require prior IBC review and approval (see Section III–B–5).

Experiments involving the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates (see Appendix F).

Appendix C–V—Extrachromosomal Elements of Gram Positive Organisms

Recombinant DNA molecules derived entirely from extrachromosomal elements of the organisms listed below (including shuttle vectors constructed from vectors described in Appendix C), propagated and maintained in organisms listed below are exempt from these Guidelines.

Bacillus subtilis
Bacillus pumilus
Bacillus licheniformis
Bacillus thuringiensis
Bacillus cereus
Bacillus amyloliquefaciens
Bacillus brevis
Bacillus natto
Bacillus niger
Bacillus aterrimus
Bacillus amylosaccharliticus
Bacillus anthracis
Bacillus globigii
Bacillus megaterium
Staphylococcus aureus
Staphylococcus epidermidis
Staphylococcus carnosus
Clostridium acetobutylicom
Pediococcus damnosus
Pediococcus pentosaceus
Pediococcus acidilactici
Lactobacillus casei
Listeria grayi
Listeria murrayi
Listeria monocytogenes
Streptococcus pyogenes
Streptococcus agalactiae
Streptococcus sanguis
Streptococcus salivarious
Streptococcus cremoris
Streptococcus pneumoniae
Streptococcus avium
Streptococcus faecalis
Streptococcus anginosus
Streptococcus sobrinus
Streptococcus lactis
Streptococcus mutans
Streptococcus equisimilis
Streptococcus thermophylus

Streptococcus milleri
Streptococcus durans
Streptococcus mitior
Streptococcus ferus

Exceptions. Experiments described in Section III–A which require specific RAC review and NIH approval before initiation of the experiment.

Large-scale experiments (e.g., more than 10 liters of culture) require prior IBC review and approval (see Section III–B–5).

Experiments involving the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates (see Appendix F).

Appendix C–VI—Footnotes and References of Appendix C

1. The original reference to organisms as Class 1, 2, 3, 4, or 5 refers to the classification in the publication *Classification of Etiologic Agents on the Basis of Hazard*, 4th Edition, July 1974; U.S. Department of Health, Education and Welfare, Public Health Service, Centers for Disease Control, Office of Biosafety, Atlanta, Georgia 30333.

The Director, NIH, with advice of the Recombinant DNA Advisory Committee, may revise the classification for the purposes of these Guidelines (see Section IV–C–1–b–(2)–(d)). The revised list of organisms in each class is reprinted in Appendix B to these Guidelines.

2. A subset of non-conjugative plasmid vectors are also poorly mobilizable (e.g., pBR322, pBR313). Where practical, these vectors should be employed.

3. Defined as observable under optimal laboratory conditions by transformation, transduction, phage infection, and/or conjugation with transfer of phage, plasmid, and/or chromosomal genetic information. Note that this definition of exchange may be less stringent than that applied to exempt organisms under Section III–D–4.

4. As classified in the Third Report of the International Committee on Taxonomy of Viruses: Classification and Nomenclature of Viruses, R.E.F. Matthews, Ed. Intervirology 12 (129–296) 1979.

5. i.e., the total of all genomes within a Family shall not exceed one-half of the genome.

Appendix D—Actions Taken Under the Guidelines

As noted in the subsections of Section IV–C–1–b–(1), the Director, NIH, may take certain actions with regard to the Guidelines after the issues have been considered by the RAC. Some of the actions taken to date include the following:

Appendix D–I

Permission is granted to clone foot and mouth disease virus in the EK1 host-vector system consisting of *E. coli* K–12 and the vector pBR322, all work to be done at the Plum Island Animal Disease Center.

Appendix D–II

Certain specified clones derived from segments of the foot and mouth disease virus may be transferred from Plum Island Animal Disease Center to the facilities of Genentech, Inc., of South San Francisco, California. Further development of the clones at Genentech has been approved under BL1+EK1 conditions.

Appendix D–III

The Rd strain of *Hemophilus influenzae* can be used as a host for the propagation of the cloned Tn 10 tet R gene derived from *E. coli* K–12 employing the non-conjugative *Hemophilus* plasmid, pRSF0085, under BL1 conditions.

Appendix D–IV

Permission is granted to clone certain subgenomic segments of foot and mouth disease virus in HV1 *Bacillus subtilis* and *Saccharomyces cerevisiae* host-vector systems under BL1 conditions at Genentech, Inc., South San Francisco, California.

Appendix D–V

Permission is granted to Dr. Ronald Davis of Stanford University to field test corn plants modified by recombinant DNA techniques under specified containment conditions.

Appendix D–VI

Permission is granted to clone in *E. coli* K–12 under BL1 physical containment conditions subgenomic segments of rift valley fever virus subject to conditions which have been set forth by the RAC.

Appendix D–VII

Attenuated laboratory strains of *Salmonella typhimurium* may be used under BL1 physical containment conditions to screen for the *Saccharomyces cerevisiae* pseudouridine synthetase gene. The plasmid YEp13 will be employed as the vector.

Appendix D–VIII

Permission is granted to transfer certain clones of subgenomic segments of foot and mouth disease virus from Plum Island Animal Disease Center to the laboratories of Molecular Genetics, Inc., Minnetonka, Minnesota, and to work with these clones under BL1 containment conditions. Approval is contingent upon review of data on infectivity testing of the clones by a working group of the RAC.

Appendix D–IX

Permission is granted to Dr. John Sanford of Cornell University to field test tomato and tobacco plants transformed with bacterial (*E. coli* K–12) and yeast DNA using pollen as a vector.

Appendix D–X

Permission is granted to Drs. Steven Lindow and Nickolas Panopoulos of the University of California, Berkeley, to release under specified conditions *Pseudomonas syringae* pv. *syringae* and *Erwinia herbicola* carrying *in vitro* generated deletions of all or part of the genes involved in ice nucleation.

Appendix D–XI

Agracetus of Middleton, Wisconsin, may field test under specified conditions disease resistant tobacco plants prepared by recombinant DNA techniques.

Appendix E—Certified Host-Vector Systems

(See also Appendix I)

While many experiments using *E. coli* K–12, *Saccharomyces cerevisiae* and *Bacillus subtilis* are currently exempt from the Guidelines under Section III–D–5, some derivatives of these host-vector systems were previously classified as HV1 or HV2. A listing of those systems follows:

Appendix E–I—Bacillus subtilis

HV1. The following plasmids are accepted as the vector components of certified *B. subtilis* HV1 systems: pUB110, pC194, pS194, pSA2100, pE194, pT127, pUB112, pC221, pC223, and pAB124. *B. subtilis* strains RUB 331 and BGSC 1S53 have been certified as the host component of HV1 systems based on these plasmids.

HV2. The asporogenic mutant derivative of *Bacillus subtilis*, ASB 298, with the following plasmids as the vector component: pUB110, pC194, pS194, pSA2100, pE194, pT127, pUB112, pC221, pC223, and pAB124.

Appendix E–II—Saccharomyces cerevisiae

HV2. The following sterile strains of *Saccharomyces cerevisiae*, all of which have the ste–VC9 mutation, SHY1, SHY2, SHY3, and SHY4. The following plasmids are certified for use: YIp1, YEp2, YEp4, YIp5, YEp6, YRp7, YEp20, YEp21, YEp24, YIp25, YIp26, YIp27, YIp28, YIp29, YIp30, YIp31, YIp32, and YIp33.

Appendix E–III—Escherichia coli

EK2 Plasmid Systems. The *E. coli* K–12 strain chi–1776. The following

plasmids are certified for use: pSC101, pMB9, pBR313, pBR322, pDH24, pBR325, pBR327, pGL101, and pHB1. The following *E. coli/S. cerevisiae* hybrid plasmids are certified as EK2 vectors when used in *E. coli* chi-1776 or in the sterile yeast strains, SHY1, SHY2, SHY3, and SHY4: YIp1, YEp2, YEp4, YIp5, YEp6, YRp7, YEp20, YEp21, YEp24, YIp25, YIp26, YIp27, YIp28, YIp29, YIp30, YIp31, YIp32, and YIp33.

EK2 Bacteriophage Systems. The following are certified EK2 systems based on bacteriophage lambda:

Vector	*Host*
λgt*WES*·λB′	DP50*supF*
λgt *WES*λB★	DP50*supF*
λgt ZJ *vir*·λB′	*E. coli* K-12
λgtALO·λB	DP50*supF*
Charon 3A	DP50 or DP50*supF*
Charon 4A	DP50 or DP50*supF*
Charon 16A	DP50 or DP50*supF*
Charon 21A	DP50*supF*
Charon 23A	DP50 or DP50*supF*
Charon 24A	DP50 or DP50*supF*

E. coli K-12 strains chi-2447 and chi-2281 are certified for use with lambda vectors that are certified for use with strain DP50 or DP50*supF* provided that the su⁻ strain not be used as a propagation host.

Appendix E-IV—Neurospora crassa

HV1. The following specified strains of *Neurospora crassa* which have been modified to prevent aerial dispersion:

Inl (inositolless) strains 37102, 37401, 46316, 64001, and 89601.

Csp-2 strain UCLA37 and csp-2 strains FS 590, UCLA101 (these are conidial separation mutants).

Eas strain UCLA191 (an "easily wettable" mutant).

Appendix E-V—Streptomyces

HV1. The following *Streptomyces* species: *Streptomyces coelicolor, S. lividans, S. parvulus,* and *S. griseus.* The following are accepted as vector components of certified *Streptomyces* HV1 systems: *Streptomyces* plasmids SCP2, SLP1.2, pIJ101, actinophage phi C31, and their derivatives.

Appendix E-VI—Pseudomonas putida

HV1. Pseudomonas putida strains KT2440 with plasmid vectors pKT262, pKT263, and pKT264.

Appendix F—Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates

Appendix F-I—General Information.

Appendix F specifies the containment to be used for the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates. The cloning of genes coding for molecules toxic for vertebrates that have an LD_{50} of less than 100 nanograms per kilogram body weight (e.g., microbial toxins such as the botulinum toxins, tetanus toxin, diphtheria toxin, *Shigella dysenteriae* neurotoxin) is covered under Section III-A-1 of the Guidelines and requires RAC review and NIH and IBC approval before initiation. No specific restrictions shall apply to the cloning of genes if the protein specified by the gene has an LD_{50} of 100 micrograms or more per kilogram of body weight. Experiments involving genes coding for toxic molecules with an LD_{50} of 100 nanograms or less per kilogram body weight shall be registered with ORDA prior to initiating the experiments. A list of toxic molecules classified as to LD_{50} is available from ORDA. Testing procedures for determining toxicity of toxic molecules not on the list are available from ORDA. The results of such tests shall be forwarded to ORDA which will consult with the RAC Working Group on Toxins prior to inclusion of the molecules on the list (see Section IV-C-1-b-(2)-(e)).

Appendix F-II—Containment Conditions for Cloning of Toxic Molecule Genes in E. coli K-12

Appendix F-II-A. Cloning of genes coding for molecules toxic for vertebrates that have LD_{50} in the range of 100 nanograms to 1000 nanograms per kilogram body weight (e.g., abrin, *Clostridium perfringens* epsilon toxin) may proceed under BL2 + EK2 or BL3 + EK1 containment conditions.

Appendix F-II-B. Cloning of genes for the biosynthesis of molecules toxic for vertebrates with LD_{50} in the range of 1 microgram to 100 micrograms per kilogram body weight may proceed under BL1 + EK1 containment conditions (e.g., *Staphylococcus* enterotoxin B toxin, *Stophylococcus* alpha toxin, ricin, *Pseudomonas* exotoxin A, *Bordatella pertussis* toxin, the lethal factor of *Bacillus anthracis*, the *Pasteurella pestis* mouse toxins, the oxygen-labile hemolysins such as streptolysin O, and certain neurotoxins present in snake venoms and other venoms).

Appendix F-II-C. Some enterotoxins are substantially more toxic when administered enterally than parenterally. The following enterotoxins shall be subject to BL1 + EK1 containment conditions: cholera toxin, the heat labile toxins of *E. coli, Klebsiella,* and other related bacteria that may be identified by cross-reaction with an antiserum raised against cholera toxin, and the heat stable toxins of *E. coli* and of *Yersinia enterocolitica.*

Appendix F-III—Containment Conditions for Cloning of Toxic Molecule Genes in Organisms Other Than E. coli K-12

Requests involving the cloning of genes coding for molecules toxic for vertebrates in host-vector systems other than *E. coli* K-12 will be evaluated by ORDA which will consult with the Working Group on Toxins (see Section IV-C-1-b-(3)-(f)).

Appendix F-IV—Specific Approvals

Appendix F-IV-A. Permission is granted to clone the Exotoxin A gene of *Pseudomonas aeruginosa* under BL1 conditions in *Pseudomonas aeruginosa* and in *Pseudomonas putida.*

Appendix F-IV-B. The pyrogenic exotoxin type A (Tox A) gene of *Staphylococcus aureus* may be cloned in an HV2 *Bacillus subtilis* host-vector system under BL3 containment conditions.

Appendix F-IV-C. Restriction fragments of *Corynephage Beta* carrying the structural gene for diphtheria toxin may be safely cloned in *e. coli* K-12 in high containment Building 550 at the Frederick Cancer Research Facility. Laboratory practices and containment equipment are to be specified by the IBC. If the investigators wish to proceed with the experiments, a prior review will be conducted to advise NIH whether the proposal has sufficient scientific merit to justify the use of the NIH BL4 facility.

Appendix F-IV-D. The genes coding for the *Staphylococcus aureus* determinants, A, B, and F, which may be implicated in toxic shock syndrome may be cloned in *E. coli* K-12 under BL2 + EK1 conditions. The *Staphylococcus aureus* strain used as the donor is to be alpha toxin minus. It is suggested that, if possible, the donor *Staphylococcus aureus* strain should lack other toxins with LD_{50}s in the range of one microgram per kilogram body weight such as the exfoliative toxin.

Appendix F-IV-E. Fragments F-1, F-2, and F-3 of the diphtheria toxin gene (tox) may be cloned in *E. coli* K-12 under BL1 + EK1 containment conditions and may be cloned in *Bacillus subtilis* host-vector systems under BL1 containment conditions. Fragment F-1 and fragment F-2 both contain: (i) Some or all of the transcriptional control elements of *tox;* (ii) the signal peptide; and (iii) fragment A (the center responsible for ADP-ribosylation of elongation factor 2). Fragment F-3 codes for most of the non-toxic fragment B of the toxin and contains no sequences coding for any portion of the enzymatically active fragment A moiety.

Appendix F–IV–F. The gene(s) coding for a toxin (designated LT-like) isolated from *E. coli* which is similar to the *E. coli* heat labile enterotoxin (LT) with respect to its activities and mode of action but is not neutralized by antibodies against cholera enterotoxin or against LT from human or porcine *E. coli* strains, and sequences homologous to the *E. coli* LT-like toxin gene may be cloned under BL1+EK1 conditions.

Appendix F–IV–G. Genes from *Vibrio fluvialis, Vibrio mimicus,* and non 0–1 *Vibrio cholerae,* specifying virulence factors for animals, may be cloned under BL1+EK1 conditions. The virulence factors to be cloned will be selected by testing fluid induction in suckling mice and Y–1 mouse adrenal cells.

Appendix F–IV–H. The intact structural gene(s) of the Shiga-like toxin from bacterial species classified in the families *Enterobacteriaceae* or *Vibrionaceae* including *Campylobacter* species may be cloned in *E. coli* K-12 under BL3+EK1 containment conditions.

E. coli host-vector systems expressing the Shiga-like toxin gene product may be moved from BL3+EK1 to BL2+EK1 containment conditions provided that: (1) The amount of toxin produced by the modified host-vector systems be no greater than that produced by the positive control strain *Shigella dysenteriae* 60R, grown and measured under optimal conditions; and (2) the cloning vehicle is to be an EK1 vector preferably belonging to the class of poorly mobilizable plasmids such as pBR322, pBR328, and pBR325.

Nontoxinogenic fragments of the Shiga-like toxin structural gene(s) may be moved from BL3+EK1 to BL2+EK1 containment conditions or such nontoxic fragments may be directly cloned in *E. coli* K–12 under BL2+EK1 conditions provided that the *E. coli* host-vector systems containing the fragments do not contain overlapping fragments which together would encompass the Shigalike toxin structural gene(s).

Appendix F–IV–I. A hybrid gene in which the gene coding for the melanocyte stimulating hormone (MSH) is joined to a segment of the gene encoding diphtheria toxin may be safely propagated in *E. coli* K–12 under BL4 containment in high containment building 550 at the Frederick Cancer Research Facility. If the investigators wish to proceed with the experiment, a prior review will be conducted to advise NIH whether the proposal has sufficient scientific merit to justify the use of the NIH BL4 facility. Before any of the strains may be removed from the BL4 facility, data on their safety shall be evaluated by the Working Group in Toxins and the working group recommendation shall be acted upon by NIH.

Appendix F–IV–J. The gene segment encoding the A subunit of chlolera toxin of *Vibrio cholerae* may be joined to the transposons Tn5 and Tn5–131 and the A-subunit::Tn5–131 hybrid gene cloned in *E. coli* K–12 and *V. cholerae* under BL1 containment conditions.

Appendix F–IV–K. A hybrid gene in which the gene coding for interleukin 2 (IL–2) is joined to a specific segment of the gene encoding diphtheria toxin may be propagated in *E. coli* K–12 host-vector systems under BL2 containment plus BL3 practices, with the use of poorly mobilizable plasmid vectors such as EK2 certified plasmids.

Appendix G—Physical Containment

Appendix G–I—Standard Practices and Training

The first principle of containment is a strict adherence to good microbiological practices [1–10]. Consequently, all personnel directly or indirectly involved in experiments on recombinant DNAs must receive adequate instruction (see Sections IV–B–1–e and IV–B–5–d). This shall, as a minimum, include instructions in aseptic techniques and in the biology of the organisms used in the experiments so that the potential biohazards can be understood and appreciated.

Any research group working with agents with a known or potential biohazard shall have an emergency plan which describes the procedures to be followed if an accident contaminates personnel or the environment. The PI must ensure that everyone in the laboratory is familiar with both the potential hazards of the work and the emergency plan (see Sections IV–B–3–d and IV–B–5–e). If a research group is working with a known pathogen for which there is an effective vaccine, the vaccine should be made available to all workers. Where serological monitoring is clearly appropriate, it shall be provided (see Section IV–B–1–f).

The "Laboratory Safety Monograph" and *Biosafety in Microbiological and Biomedical Laboratories* [2] booklets describe practices, equipment, and facilities in detail.

Appendix G–II—Physical Containment Levels

The objective of physical containment is to confine organisms containing recombinant DNA molecules and thus to reduce the potential for exposure of the laboratory worker, persons outside of the laboratory, and the environment to organisms containing recombinant DNA molecules. Physical containment is achieved through the use of laboratory practices, containment equipment, and special laboratory design. Emphasis is placed on primary means of physical containment which are provided by laboratory practices and containment equipment. Special laboratory design provides a secondary means of protection against the accidental release of organisms outside the laboratory or to the environment. Special laboratory design is used primarily in facilities in which experiments of moderate to high potential hazards are performed.

Combinations of laboratory practices, containment equipment, and special laboratory design can be made to achieve different levels of physical containment. Four levels of physical containment, which are designated as BL1, BL2, BL3, and BL4, are described. It should be emphasized that the descriptions and assignments of physical containment detailed below are based on existing approaches to containment of pathogenic organisms [2]. The National Cancer Institute describes three levels for research on oncogenic viruses which roughly correspond to our BL2, BL3, and BL4 level [3].

It is recognized that several different combinations of laboratory practices, containment equipment, and special laboratory design may be appropriate for containment of specific research activities. The Guidelines, therefore, allow alternative selections of primary containment equipment within facilities that have been designed to provide BL3 and BL4 levels of physical containment. The selection of alternative methods of primary containment is dependent, however, on the level of biological containment provided by the host-vector system used in the experiment. Consideration will also be given by the Director, NIH, with the advice of the RAC to other combinations which achieve an equivalent level of containment [see Section IV–C–1–b–(2)–(b)].

Appendix G–II–A—Biosafety Level 1 (BL1) [13]

Appendix G–II–A–1. Standard Microbiological Practices.

Appendix G–II–A–1–a. Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments are in progress.

Appendix G–II–A–1–b. Work surfaces are decontaminated once a day and after any spill of viable material.

Appendix G–II–A–1–c. All contaminated liquid or solid wastes are decontaminated before disposal.

Appendix G–II–A–1–d. Mechanical pipetting devices are used; mouth pipetting is prohibited.

Appendix G–II–A–1–e. Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Food may be stored in cabinets or refrigerators designated and used for this purpose only.

Appendix G–II–A–1–f. Persons wash their hands after they handle materials involving organisms containing recombinant DNA molecules, and animals, and before leaving the laboratory.

Appendix G–II–A–1–g. All procedures are performed carefully to minimize the creation of aerosols.

Appendix G–II–A–1–h. It is recommended that laboratory coats, gowns, or uniforms be worn to prevent contamination or soiling of street clothes.

Appendix G–II–A–2—Special Practices

Appendix G–II–A–2–a. Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leakproof container which is closed before being removed from the laboratory.

Appendix G–II–A–2–b. An insect and rodent control program is in effect.

Appendix G–II–A–3—Containment Equipment

Appendix G–II–A–3–a. Special containment equipment is generally not required for manipulations of agents assigned to Biosafety Level 1.

Appendix G–II–A–4—Laboratory Facilities

Appendix G–II–A–4–a. The laboratory is designed so that it can be easily cleaned.

Appendix G–II–A–4–b. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix G–II–A–4–c. Laboratory furniture is sturdy. Spaces between benches, cabinets, and equipment are accessible for cleaning.

Appendix G–II–A–4–d. Each laboratory contains a sink for hand-washing.

Appendix G–II–A–4–e. If the laboratory has windows that open, they are fitted with fly screens.

Appendix G–II–B—Biosafety Level 2 (BL2) [14]

Appendix G–II–B–1. Standard Microbiological Practices.

Appendix G–II–B–1–a. Access to the laboratory is limited or restricted by the laboratory director when work with organisms containing recombinant DNA molecules is in progress.

Appendix G–II–B–1–b. Work surfaces are decontaminated at least once a day and after any spill of viable material.

Appendix G–II–B–1–c. All contaminated liquid or solid wastes are decontaminated before disposal.

Appendix G–II–B–1–d. Mechanical pipetting devices are used; mouth pipetting is prohibited.

Appendix G–II–B–1–e. Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Food may be stored in cabinets or refrigerators designated and used for this purpose only.

Appendix G–II–B–1–f. Persons wash their hands after handling materials involving organisms containing recombinant DNA molecules, and animals, and when they leave the laboratory.

Appendix G–II–B–1–g. All procedures are performed carefully to minimize the creation of aerosols.

Appendix G–II–B–1–h. Experiments of lesser biohazard potential can be carried out concurrently in carefully demarcated areas of the same laboratory.

Appendix G–II–B–2—Special Practices

Appendix G–II–B–2–a. Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leakproof container which is closed before being removed from the laboratory.

Appendix G–II–B–2–b. The laboratory director limits access to the laboratory. The director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.

Appendix G–II–B–2–c. The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet any specific entry requirements (e.g., immunization) enter the laboratory or animal rooms.

Appendix G–II–B–2–d. When the organisms containing recombinant DNA molecules in use in the laboratory require special provisions for entry (e.g., vaccination), a hazard warning sign incorporating the universal biohazard symbol is posted on the access door to the laboratory work area. The hazard warning sign identifies the agent, lists the name and telephone number of the laboratory director or other responsible person(s), and indicates the special requirement(s) for entering the laboratory.

Appendix G–II–B–2–e. An insect and rodent control program is in effect.

Appendix G–II–B–2–f. Laboratory coats, gowns, smocks, or uniforms are worn while in the laboratory. Before leaving the laboratory for nonlaboratory areas (e.g., cafeteria, library, administrative offices), this protective clothing is removed and left in the laboratory or covered with a clean coat not used in the laboratory.

Appendix G–II–B–2–g. Animals not involved in the work being performed are not permitted in the laboratory.

Appendix G–II–B–2–h. Special care is taken to avoid skin contamination with organisms containing recombinant DNA molecules; gloves should be worn when handling experimental animals and when skin contact with the agent is unavoidable.

Appendix G–II–B–2–i. All wastes from laboratories and animal rooms are appropriately decontaminated before disposal.

Appendix G–II–B–2–j. Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for the injection or aspiration of fluids containing organisms that contain recombinant DNA molecules. Extreme caution should be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Needles should not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

Appendix G–II–B–2–k. Spills and accidents which result in overt exposures to organisms containing recombinant DNA molecules are immediately reported to the laboratory director. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

Appendix G–II–B–2–l. When appropriate, considering the agent(s) handled, baseline serum samples for laboratory and other at-risk personnel are collected and stored. Additional serum specimens may be collected periodically depending on the agents handled or the function of the facility.

Appendix G–II–B–2–m. A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read

instructions on practices and procedures and to follow them.

Appendix G–II–B–3—Containment Equipment

Appendix G–II–B–3–a. Biological safety cabinets (Class I or II) (see Appendix G–III–12) or other appropriate personal protective or physical containment devices are used whenever:

Appendix G–II–B–3–a–(1). Procedures with a high potential for creating aerosols are conducted [15]. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of materials whose internal pressures may be different from ambient pressures, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.

Appendix G–II–B–3–a–(2). High concentrations or large volumes of organisms containing recombinant DNA molecules are used. Such materials may be centrifuged in the open laboratory if sealed heads or centrifuge safety cups are used and if they are opened only in a biological safety cabinet.

Appendix G–II–B–4—Laboratory Facilities

Appendix G–II–B–4–a. The laboratory is designed so that it can be easily cleaned.

Appendix G–II–B–4–b. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix G–II–B–4–c. Laboratory furniture is sturdy and spaces between benches, cabinets, and equipment are accessible for cleaning.

Appendix G–II–B–4–d. Each laboratory contains a sink for hand-washing.

Appendix G–II–B–4–e. If the laboratory has windows that open, they are fitted with fly screens.

Appendix G–II–B–4–f. An autoclave for decontaminating laboratory wastes is available.

Appendix G–II–C—Biosafety Level 3 (BL3) [16]

Appendix G–II–C–1. Standard Microbiological Practices.

Appendix G–II–C–1–a. Work surfaces are decontaminated at least once a day and after any spill of viable material.

Appendix G–II–C–1–b. All contaminated liquid or solid wastes are decontaminated before disposal.

Appendix G–II–C–1–c. Mechanical pipetting devices are used; mouth pipetting is prohibited.

Appendix G–II–C–1–d. Eating, drinking, smoking, storing food, and applying cosmetics are not permitted in the work area.

Appendix G–II–C–1–e. Persons wash their hands after handling materials involving organisms containing recombinant DNA molecules, and animals, and when they leave the laboratory.

Appendix G–II–C–1–f. All procedures are performed carefully to minimize the creation of aerosols.

Appendix G–II–C–1–g. Persons under 16 years of age shall not enter the laboratory.

Appendix G–II–C–1–h. If experiments involving other organisms which require lower levels of containment are to be conducted in the same laboratory concurrently with experiments requiring BL3 level physical containment, they shall be conducted in accordance with all BL3 level laboratory practices.

Appendix G–II–C–2—Special Practices

Appendix G–II–C–2–a. Laboratory doors are kept closed when experiments are in progress.

Appendix G–II–C–2–b. Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leakproof container which is closed before being removed from the laboratory.

Appendix G–II–C–2–c. The laboratory director controls access to the laboratory and restricts access to persons whose presence is required for program or support purposes. The director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.

Appendix G–II–C–2–d. The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential biohazard, who meet any specific entry requirements (e.g., immunization), and who comply with all entry and exit procedures enter the laboratory or animal rooms.

Appendix G–II–C–2–e. When organisms containing recombinant DNA molecules or experimental animals are present in the laboratory or containment module, a hazard warning sign incorporating the universal biohazard symbol is posted on all laboratory and animal room access doors. The hazard warning sign identifies the agent, lists the name and telephone number of the laboratory director or other responsible person(s), and indicates any special requirements for entering the laboratory, such as the need for immunizations, respirators, or other personal protective measures.

Appendix G–II–C–2–f. All activities involving organisms containing recombinant DNA molecules are conducted in biological safety cabinets or other physical containment devices within the containment module. No work in open vessels is conducted on the open bench.

Appendix G–II–C–2–g. The work surfaces of biological safety cabinets and other containment equipment are decontaminated when work with organisms containing recombinant DNA molecules is finished. Plastic-backed paper toweling used on nonperforated work surfaces within biological safety cabinets facilitates clean-up.

Appendix G–II–C–2–h. An insect and rodent program is in effect.

Appendix G–II–C–2–i. Laboratory clothing that protects street clothing (e.g., solid front or wrap-around gowns, scrub suits, coveralls) is worn in the laboratory. Laboratory clothing is not worn outside the laboratory, and it is decontaminated before being laundered.

Appendix G–II–C–2–j. Special care is taken to avoid skin contamination with contaminated materials; gloves should be worn when handling infected animals and when skin contact with infectious materials is unavoidable.

Appendix G–II–C–2–k. Molded surgical masks or respirators are worn in rooms containing experimental animals.

Appendix G–II–C–2–l. Animals and plants not related to the work being conducted are not permitted in the laboratory.

Appendix G–II–C–2–m. Laboratory animals held in a BL3 area shall be housed in partial-containment caging systems, such as Horsfall units [11], open cages placed in ventilated enclosures, solid-wall and -bottom cages covered by filter bonnets, or solid-wall and -bottom cages placed on holding racks equipped with ultraviolet in radiation lamps and reflectors.

Note.—Conventional caging systems may be used provided that all personnel wear appropriate personal protective devices. These shall include at a minimum wrap-around gowns, head covers, gloves, shoe covers, and respirators. All personnel shall shower on exit from areas where these devices are required.

Appendix G–II–C–2–n. All wastes from laboratories and animal rooms are appropriately decontaminated before disposal.

Appendix G–II–C–2–o. Vacuum lines are protected with high efficiency particulate air (HEPA) filters and liquid disinfectant traps.

Appendix G–II–C–2–p. Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and

or vapor methods in an airlock or chamber designed for this purpose.

Appendix G–II–D–2–c. Only persons whose presence in the facility or individual laboratory rooms is required for program or support purposes are authorized to enter. The supervisor has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory. Access to the facility is limited by means of secure, locked doors; accessibility is managed by the laboratory director, biohazards control officer, or other person responsible for the physical security of the facility. Before entering, persons are advised of the potential biohazards and instructed as to appropriate safeguards for ensuring their safety. Authorized persons comply with the instructions and all other applicable entry and exit procedures. A logbook signed by all personnel indicates the date and time of each entry and exit. Practical and effective protocols for emergency situations are established.

Appendix G–II–D–2–d. Personnel enter and leave the facility only through the clothing change and shower rooms. Personnel shower each time they leave the facility. Personnel use the airlocks to enter or leave the laboratory only in an emergency.

Appendix G–II–D–2–e. Street clothing is removed in the outer clothing change room and kept there. Complete laboratory clothing, including undergarments, pants and shirts or jumpsuits, shoes, and gloves, is provided and used by all personnel entering the facility. Head covers are provided for personnel who do not wash their hair during the exit shower. When leaving the laboratory and before proceeding into the shower area, personnel remove their laboratory clothing and store it in a locker or hamper in the inner change room.

Appendix G–II–D–2–f. When materials that contain organisms containing recombinant DNA molecules or experimental animals are present in the laboratory or animal rooms, a hazard warning sign incorporating the universal biohazard symbol is posted on all access doors. The sign identifies the agent, lists the name of the laboratory director or other responsible person(s), and indicates any special requirements for entering the area (e.g., the need for immunizations or respirators).

Appendix G–II–D–2–g. Supplies and materials needed in the facility are brought in by way of the double-doored autoclave, fumigation chamber, or airlock which is appropriately decontaminated between each use. After securing the outer doors, personnel within the facility retrieve the materials by opening the interior doors or the autoclave, fumigation chamber, or airlock. These doors are secured after materials are brought into the facility.

Appendix G–II–D–2–h. An insect and rodent control program is in effect.

Appendix G–II–D–2–i. Materials (e.g., plants, animals, and clothing) not related to the experiment being conducted are not permitted in the facility.

Appendix G–II–D–2–j. Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral part of unit) are used for the injection or aspiration of fluids containing organisms that contain recombinant DNA molecules. Needles should not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe following use. The needle and syringe should be placed in a puncture-resistant container and decontaminated, preferably by autoclaving before [illegible]. Whenever possible, [illegible] are used instead of sharp needles for [illegible] gavage).

Appendix G–II–D–2–k. A system is set up for reporting laboratory accidents and exposures and employee absenteeism and for the medical surveillance of potential laboratory-associated illnesses. Written records are prepared and maintained. An essential adjunct to such a reporting surveillance system is the availability of a facility for quarantine, isolation, and medical care of personnel with potential or known laboratory associated illnesses.

Appendix G–II–D–2–l. Laboratory animals involved in experiments requiring BL4 level physical containment shall be housed either in cages contained in Class III cabinets or in partial containment [illegible] (such as Horsfall units) [illegible] cages placed in ventilated racks [illegible] or solid-wall and -bottom [illegible] holding racks equipped [illegible] ultraviolet irradiation lamps and [illegible] that are located in a specially designed area in which all personnel are required to wear one-piece positive pressure suits.

Appendix G–II–D–3.—Alternative Selection of Containment Equipment. Experimental procedures involving a host-vector system that provides a one-step higher level of biological containment than that specified can be conducted in the BL4 facility using containment equipment requirements specified for the BL3 level of physical containment. Alternative combinations of containment safeguards are shown in Table I.

Appendix G–II–D–3.—Containment Equipment

Appendix G–II–D–3–a. All procedures within the facility with agents assigned to Biosafety Level 4 are conducted in the Class III biological safety cabinet or in Class I or II biological safety cabinets used in conjunction with one-piece positive pressure personnel suits ventilated by a life-support system.

Appendix G–II–D–4.—Laboratory Facilities

Appendix G–II–D–4–a. The maximum containment facility consists of either a separate building or a clearly demarcated and isolated zone within a building. Outer and inner change rooms separated by a shower are provided for personnel entering and leaving the facility. A double-doored autoclave, fumigation chamber, or ventilated airlock is provided for passage of those materials, supplies, or equipment which are not brought into the facility through the change room.

Appendix G–II–D–4–b. Walls, floors, and ceilings of the facility are constructed to form a sealed internal shell which facilitates fumigation and is animal and insect proof. The internal surfaces of this shell are resistant to liquids and chemicals, thus facilitating cleaning and decontamination of the area. All penetrations in these structures and surfaces are sealed. Any drains in the floors contain traps filled with a chemical disinfectant of demonstrated efficacy against the target agent, and they are connected directly to the liquid waste decontamination system. Sewer and other ventilation lines contain HEPA filters.

Appendix G–II–D–4–c. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize the horizontal surface area on which dust can settle.

Appendix G–II–D–4–d. Bench tops have seamless surfaces which are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix G–II–D–4–e. Laboratory furniture is of simple and sturdy construction, and spaces between benches, cabinets, and equipment are accessible for cleaning.

Appendix G–II–D–4–f. A foot, elbow, or automatically operated hand-washing sink is provided near the door of each laboratory room in the facility.

Appendix G–II–D–4–g. If there is a central vacuum system, it does not serve areas outside the facility. In-line HEPA filters are placed as near as practicable to each use point or service cock. Filters

or vapor methods in an airlock or chamber designed for this purpose.

Appendix G–II–D–2–c. Only persons whose presence in the facility or individual laboratory rooms is required for program or support purposes are authorized to enter. The supervisor has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory. Access to the facility is limited by means of secure, locked doors; accessibility is managed by the laboratory director, biohazards control officer, or other person responsible for the physical security of the facility. Before entering, persons are advised of the potential biohazards and instructed as to appropriate safeguards for ensuring their safety. Authorized persons comply with the instructions and all other applicable entry and exit procedures. A logbook signed by all personnel indicates the date and time of each entry and exit. Practical and effective protocols for emergency situations are established.

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Appendix G–II–D–2–f. When materials that contain organisms containing recombinant DNA molecules or experimental animals are present in the laboratory or animal rooms, a hazard warning sign incorporating the universal biohazard symbol is posted on all access doors. The sign identifies the agent, lists the name of the laboratory director or other responsible person(s), and indicates any special requirements for entering the area (e.g., the need for immunizations or respirators).

Appendix G–II–D–2–g. Supplies and materials needed in the facility are brought in by way of the double-doored autoclave, fumigation chamber, or airlock which is appropriately decontaminated between each use. After securing the outer doors, personnel within the facility retrieve the materials by opening the interior doors or the autoclave, fumigation chamber, or airlock. These doors are secured after materials are brought into the facility.

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Appendix G–II–D–2–j. Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral part of unit) are used for the injection or aspiration of fluids containing organisms that contain recombinant DNA molecules. Needles should not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe following use. The needle and syringe should be placed in a puncture-resistant container and decontaminated, preferably by autoclaving before discard or reuse. Whenever possible, cannulas are used instead of sharp needles (e.g., gavage).

Appendix G–II–D–2–k. A system is set up for reporting laboratory accidents and exposures and employee absenteeism and for the medical surveillance of potential laboratory-associated illnesses. Written records are prepared and maintained. An essential adjunct to such a reporting-surveillance system is the availability of a facility for quarantine, isolation, and medical care of personnel with potential or known laboratory associated illnesses.

Appendix G–II–D–2–l. Laboratory animals involved in experiments requiring BL4 level physical containment shall be housed either in cages contained in Class III cabinets or in partial containment caging systems [such as Horsfall units [11]], open cages placed in ventilated enclosures, or solid-wall and -bottom cages placed on holding racks equipped with ultraviolet irradiation lamps and reflectors that are located in a specially designed area in which all personnel are required to wear one-piece positive pressure suits.

Appendix G–II–D–2–m. Alternative Selection of Containment Equipment. Experimental procedures involving a host-vector system that provides a one-step higher level of biological containment than that specified can be conducted in the BL4 facility using containment equipment requirements specified for the BL3 level of physical containment. Alternative combinations of containment safeguards are shown in Table I.

Appendix G–II–D–3.—Containment Equipment

Appendix G–II–D–3–a. All procedures within the facility with agents assigned to Biosafety Level 4 are conducted in the Class III biological safety cabinet or in Class I or II biological safety cabinets used in conjunction with one-piece positive pressure personnel suits ventilated by a life-support system.

Appendix G–II–D–4.—Laboratory Facilities

Appendix G–II–D–4–a. The maximum containment facility consists of either a separate building or a clearly demarcated and isolated zone within a building. Outer and inner change rooms separated by a shower are provided for personnel entering and leaving the facility. A double-doored autoclave, fumigation chamber, or ventilated airlock is provided for passage of those materials, supplies, or equipment which are not brought into the facility through the change room.

Appendix G–II–D–4–b. Walls, floors, and ceilings of the facility are constructed to form a sealed internal shell which facilitates fumigation and is animal and insect proof. The internal surfaces of this shell are resistant to liquids and chemicals, thus facilitating cleaning and decontamination of the area. All penetrations in these structures and surfaces are sealed. Any drains in the floors contain traps filled with a chemical disinfectant of demonstrated efficacy against the target agent, and they are connected directly to the liquid waste decontamination system. Sewer and other ventilation lines contain HEPA filters.

Appendix G–II–D–4–c. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize the horizontal surface area on which dust can settle.

Appendix G–II–D–4–d. Bench tops have seamless surfaces which are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix G–II–D–4–e. Laboratory furniture is of simple and sturdy construction, and spaces between benches, cabinets, and equipment are accessible for cleaning.

Appendix G–II–D–4–f. A foot, elbow, or automatically operated hand-washing sink is provided near the door of each laboratory room in the facility.

Appendix G–II–D–4–g. If there is a central vacuum system, it does not serve areas outside the facility. In-line HEPA filters are placed as near as practicable to each use point or service cock. Filters

are installed to permit in-place decontamination and replacement. Other liquid and gas services to the facility are protected by devices that prevent backflow.

Appendix G–II–D–4–h. If water fountains are provided, they are foot-operated and are located in the facility corridors outside the laboratory. The water service to the fountain is not connected to the backflow-protected distribution system supplying water to the laboratory areas.

Appendix G–II–D–4–i. Access doors to the laboratory are self-closing and lockable.

Appendix G–II–D–4–j. Any windows are breakage resistant.

Appendix G–II–D–4–k. A double-doored autoclave is provided for decontaminating materials passing out of the facility. The autoclave door which opens to the area external to the facility is sealed to the outer wall and automatically controlled so that the outside door can only be opened after the autoclave "sterilization" cycle has been completed.

Appendix G–II–D–4–l. A pass-through dunk tank, fumigation chamber, or an equivalent decontamination method is provided so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the facility.

Appendix G–II–D–4–m. Liquid effluents from laboratory sinks, biological safety cabinets, floors, and autoclave chambers are decontaminated by heat treatment before being released from the maximum containment facility. Liquid wastes from shower rooms and toilets may be decontaminated with chemical disinfectants or by heat in the liquid waste decontamination system. The procedure used for heat decontamination of liquid wastes is evaluated mechanically and biologically by using a recording thermometer and an indicator microorganism with a defined heat susceptibility pattern. If liquid wastes from the shower room are decontaminated with chemical disinfectants, the chemical used is of demonstrated efficacy against the target or indicator microorganisms.

Appendix G–II–D–4–n. An individual supply and exhaust air ventilation system is provided. The system maintains pressure differentials and directional airflow as required to assure flows inward from areas outside of the facility toward areas of highest potential risk within the facility. Manometers are used to sense pressure differentials between adjacent areas maintained at different pressure levels. If a system malfunctions, the manometers sound an alarm. The supply and exhaust airflow is interlocked to assure inward (or zero) airflow at all times.

Appendix G–II–D–4–o. The exhaust air from the facility is filtered through HEPA filters and discharged to the outside so that it is dispersed away from occupied buildings and air intakes. Within the facility, the filters are located as near the laboratories as practicable in order to reduce the length of potentially contaminated air ducts. The filter chambers are designed to allow *in situ* decontamination before filters are removed and to facilitate certification testing after they are replaced. Coarse filters and HEPA filters are provided to treat air supplied to the facility in order to increase the lifetime of the exhaust HEPA filters and to protect the supply air system should air pressures become unbalanced in the laboratory.

Appendix G–II–D–4–p. The treated exhaust air from Class I and II biological safety cabinets can be discharged into the laboratory room environment or the outside through the facility air exhaust system. If exhaust air from Class I or II biological safety cabinets is discharged into the laboratory the cabinets are tested and certified at 3-month intervals. *The exhaust air from Class III biological safety cabinets is discharged, without recirculation through two sets of HEPA filters in series, via the facility exhaust air system.* If the treated exhaust air from any of these cabinets is discharged to the outside through the facility exhaust air system, it is connected to this system in a manner (e.g., thimble unit connection [12]) that avoids any interference with the air balance of the cabinets or the facility exhaust air system.

Appendix G–II–D–4–q. A specially designed suit area may be provided in the facility. Personnel who enter this area wear a one-piece positive pressure suit that is ventilated by a life-support system. The life-support system includes alarms and emergency backup breathing air tanks. Entry to this area is through an airlock fitted with airtight doors. A chemical shower is provided to decontaminate the surface of the suit before the worker leaves the area. The exhaust air from the suit area is filtered by two sets of HEPA filters installed in series. A duplicate filtration unit, exhaust fan, and an automatically starting emergency power source are provided. The air pressure within the suit area is lower than that of any adjacent area. Emergency lighting and communication systems are provided. All penetrations into the internal shell of the suit area are sealed. A double-doored autoclave is provided for decontaminating waste materials to be removed from the suit area.

TABLE 1.—POSSIBLE ALTERNATE COMBINATIONS OF PHYSICAL AND BIOLOGICAL CONTAINMENT SAFEGUARDS

Classification of physical and biological containment	Alternate physical containment			Alternate biological containment
	Laboratory facilities	Laboratory practices	Containment equipment	
BL3/HV2	BL3	BL3	BL3	HV2
	BL3	BL3	BL4	HV1
BL3/HV1	BL3	BL3	BL3	HV1
	BL3	BL3	BL2	HV2
BL4/HV1	BL4	BL4	BL4	HV1
	BL4	BL4	BL3	HV2

Appendix G–III—Footnotes and References of Appendix G

1. *Laboratory Safety at the Center for Disease Control* (Sept. 1974). U.S. Department of Health Education and Welfare Publication No. CDC 75–8116.

2. *Biosafety in Microbiological and Biomedical Laboratories*, 1st Edition (March 1984). U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, Atlanta, Georgia 30333, and National Institutes of Health, Bethesda, Maryland 20205.

3. *National Cancer Institute Safety Standards for Research Involving Oncogenic Viruses* (Oct. 1974). U.S. Department of Health, Education and Welfare Publication No. (NIH) 75–790.

4. *National Institutes of Health Biohazards Safety Guide* (1974). U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health. U.S. Government Printing Office, Stock No. 1740–00388.

5. *Biohazards in Biological Research* (1973). A. Hellman, M.N. Oxman, and R. Pollack (ed.) Cold Spring Harbor Laboratory.

6. *Handbook of Laboratory Safety* (1971). 2nd Edition. N.V. Steere (ed.). The Chemical Rubber Co., Cleveland.

7. Bodily, J.L. (1970). *General Administration of the Laboratory*. H.L. Bodily, E.L. Updyke, and J.O. Mason (eds.), Diagnostic Procedures for Bacterial, Mycotic and Parasitic Infections. American Public Health Association, New York, pp. 11–28.

8. Darlow, H.M. (1969). *Safety in the Microbiological Laboratory*. In J.R. Norris and D.W. Robbins (ed.), Methods in Microbiology. Academic Press, Inc., New York, pp. 169–204.

9. *The Prevention of Laboratory Acquired Infection* (1974). C.H. Collins, E.G. Hartley, and R. Pilsworth. Public Health Laboratory Service, Monograph Series No. 6.

10. Chatigny, M.A. (1961). *Protection Against Infection in the Microbiological Laboratory: Devices and Procedures*. In W.W. Umbreit (ed.), Advances in Applied Microbiology. Academic Press, New York, N.Y. 3:131–192.

11. Horsfall, F.L., Jr., and J.H. Baner (1940). *Individual Isolation of Infected Animals in a Single Room*. J. Bact. *40*, 569–580.

12. Biological safety cabinets referred to in this section are classified as *Class I, Class II,* or *Class III* cabinets. A *Class I* is a ventilated cabinet for personnel protection having an

inward flow of air away from the operator. The exhaust air from this cabinet is filtered through a high-efficiency particulate air (HEPA) filter. This cabinet is used in three operational modes: (1) with a full-width open front, (2) with an installed front closure panel (having four 8-inch diameter openings) without gloves, and (3) with an installed front closure panel equipped with arm-length rubber gloves. The face velocity of the inward flow of air through the full-width open front is 75 feet per minute or greater.

A *Class II* cabinet is a ventilated cabinet for personnel and product protection having an open front with inward air flow for personnel protection, and HEPA filtered mass recirculated air flow for product protection. The cabinet exhaust air is filtered through a HEPA filter. The face velocity of the inward flow of air through the full-width open front is 75 feet per minute or greater. Design and performance specifications for *Class II* cabinets have been adopted by the National Sanitation Foundation, Ann Arbor, Michigan. A *Class III* cabinet is a closed-front ventilated cabinet of gas-tight construction which provides the highest level of personnel protection of all biohazard safety cabinets. The interior of the cabinet is protected from contaminants exterior to the cabinet. The cabinet is fitted with arm-length rubber gloves and is operated under a negative pressure of at least 0.5 inches water gauge. All supply air is filtered through HEPA filters. Exhaust air is filtered through two HEPA filters or one HEPA filter and incinerator before being discharged to the outside environment. National Sanitation Foundation Standard 49. 1976. Class II (Laminar Flow) Biohazard Cabinetry. Ann Arbor, Michigan.

13. *Biosafety Level 1 is suitable for work* involving agents of no known or minimal potential hazard to laboratory personnel and the environment. The laboratory is not separated from the general traffic patterns in the building. Work is generally conducted on open bench tops. Special containment equipment is not required or generally used. Laboratory personnel have specific training in the procedures conducted in the laboratory and are supervised by a scientist with general training in microbiology or a related science (see Appendix G–III–2).

14. Biosafety Level 2 is similar to Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs in that: (1) laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists; (2) access to the laboratory is limited when work is being conducted; and (3) certain procedures in which infectious aerosols are created are conducted in biological safety cabinets or other physical containment equipment (see Appendix G–III–2).

15. Office of Research Safety, National Cancer Institute, and the Special Committee of Safety and Health Experts. 1978. "Laboratory Safety Monograph: A Supplement to the NIH Guidelines for *Recombinant DNA Research*." Bethesda, Maryland, National Institutes of Health.

16. Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents which may cause serious or potentially lethal disease as a result of exposure by the inhalation route. Laboratory personnel have specific training in handling pathogenic and potentially lethal agents and are supervised by competent scientists who are experienced in working with these agents. All procedures involving the manipulation of infectious material are conducted within biological safety cabinets or other physical containment devices or by personnel wearing appropriate personal protective clothing and devices. The laboratory has special engineering and design features. It is recognized, however, that many existing facilities may not have all the facility safeguards recommended for Biosafety Level 3 (e.g., access zone, sealed penetrations, and directional airflow, etc.). In these circumstances, acceptable safety may be achieved for routine or repetitive operations (e.g., diagnostic procedures involving the propagation of an agent for identification, typing, and susceptibility testing) in laboratories where facility features satisfy *Biosafety Level 2 recommendations* provided the recommended "Standard Microbiological Practices," "Special Practices," and "Containment Equipment" for Biosafety Level 3 are rigorously followed. The decision to implement this modification of Biosafety Level 3 recommendations should be made only by the laboratory director (see Appendix *G–III–2*).

Appendix H—Shipment

Recombinant DNA molecules contained in an organism or virus shall be shipped only as an etiologic agent under requirements of the U.S. Public Health Service, and the U.S. Department of Transportation (§ 72.3, Part 72, Title 42, and §§ 173.386–.388, Part 173, Title 49, U.S. Code of Federal Regulations (CFR)) as specified below:

Appendix H–I

Recombinant DNA molecules contained in an organism or virus requiring BL1, BL2, or BL3 physical containment, when offered for transportation or transported, are subject to all requirements of §§ 72.3(a)–(e), Part 72, Title 42 CFR, and §§ 173.386–.388, Part 173, Title 49 CFR.

Appendix H–II

Recombinant DNA molecules contained in an organism or virus requiring BL4 physical containment, when offered for transportation or transported, are subject to the requirements listed above under Appendix H–I and are also subject to § 72.3(f), Part 72, Title 42 CFR.

Appendix H–III

Information on packaging and labeling of etiologic agents is shown in Figures 1, 2, and 3. Additional information on packaging and shipment is given in the "Laboratory Safety Monograph—A Supplement to the NIH Guidelines for Recombinant DNA Research," available from the Office of Recombinant DNA Activities and in *Biosafety in Microbiological and Biomedical Laboratories* (see Appendix G–III–2).

BILLING CODE 4140-01-M

PACKAGING AND LABELING OF ETIOLOGIC AGENTS

The Interstate Shipment of Etiologic Agents (42 CFR, Part 72) was revised July 21, 1980 to provide for packaging and labeling requirements for etiologic agents and certain other materials shipped in interstate traffic.

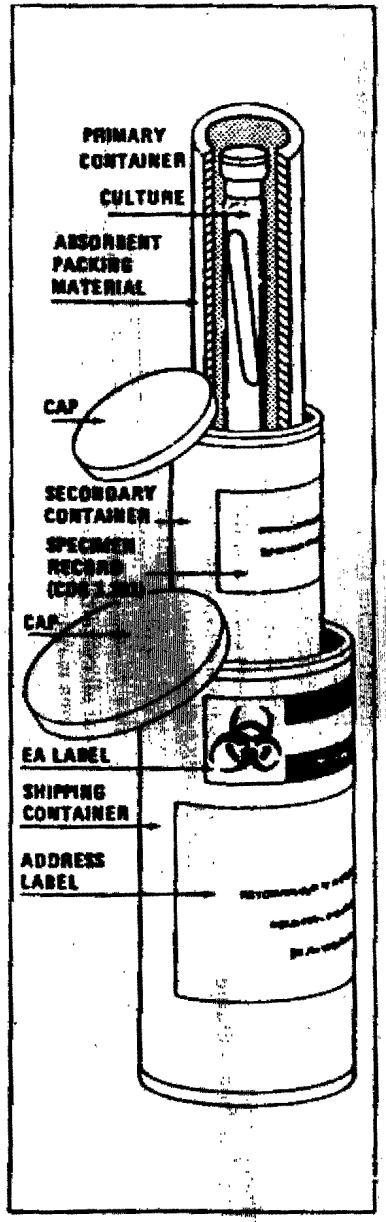
Figures 1 and 2 diagram the packaging and labeling of etiologic agents in volumes of less than 50 ml. in accordance with the provisions of subparagraph 72.3 (a) of the cited regulation. Figure illustrates the color and size of the label, described in subparagraph 72.3 (d) (1 - 5) of the regulations, which shall be affixed to all shipments of etiologic agents.

For further information on any provision of this regulation contact:

Centers for Disease Control
Attn: Biohazards Control Office
1600 Clifton Road
Atlanta, Georgia 30333

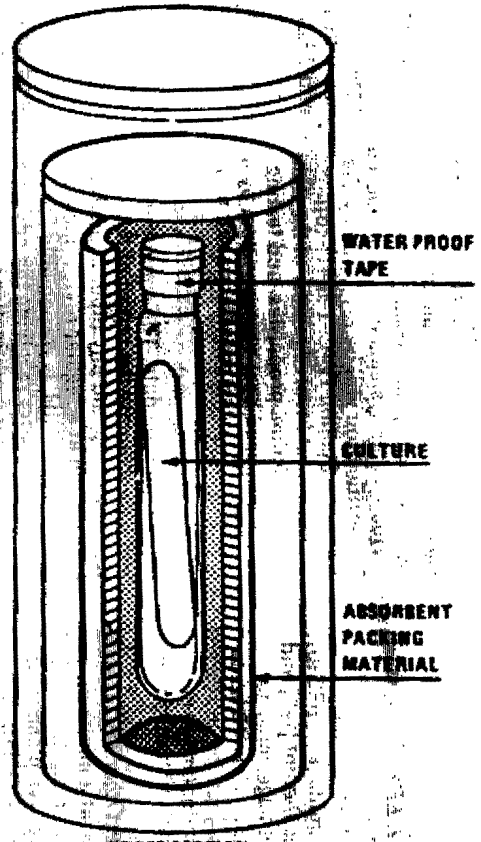
Telephone: 404–329-3883
FTS–236-3883

FIGURE 1



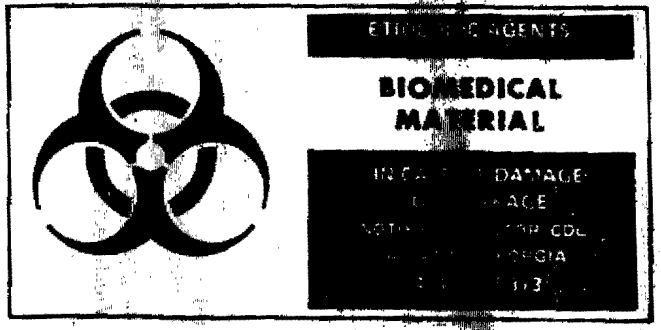
BILLING CODE 4160-61-C

FIGURE 2



CROSS SECTION OF PROPER PACKING

FIGURE 3



Appendix I—Biological Containment

(See also Appendix E)

Appendix I–I—Levels of Biological Containment.

In consideration of biological containment, the vector (plasmid, organelle, or virus) for the recombinant DNA and the host (bacterial, plant, or animal cell) in which the vector is propagated in the laboratory will be considered together. Any combination of vector and host which is to provide biological containment must be chosen or constructed so that the following types of "escape" are minimized: (i) Survival of the vector in its host outside the laboratory, and (ii) transmission of the vector from the propagation host to other nonlaboratory hosts.

The following levels of biological containment (HV, or Host-Vector, systems) for prokaryotes will be established; specific criteria will depend on the organisms to be used.

Appendix I–I–A. HV1. A host-vector system which provides a moderate level of containment. *Specific systems* are:

Appendix I–I–A–1. EK1. The host is always *E. coli* K–12 or a derivative thereof, and the vectors include nonconjugative plasmids (e.g., pSC101, ColEI, or derivatives thereof [1–7] and variants of bacteriophage, such as lambda [8–15]. The *E. coli* K–12 hosts shall not contain conjugation-proficient plasmids, whether autonomous or integrated, or generalized transducing phages.

Appendix I–I–A–2. Other HV1. Hosts and vectors shall be, at a minimum, comparable in containment to *E. coli* K–12 with a non conjugative plasmid or bacteriophage vector. The data to be considered and a mechanism for approval of such HV1 systems are described below (Appendix I–II).

Appendix I–I–B. HV2. These are host-vector systems shown to provide a high level of biological containment as demonstrated by data from suitable tests performed in the laboratory. Escape of the recombinant DNA either via survival of the organisms or via transmission of recombinant DNA to other organisms should be less than $1/10^8$ under specified conditions. Specific systems are:

Appendix I–I–B–1. For EK2 host-vector systems in which the vector is a plasmid, no more than one in 10^8 host cells should be able to perpetuate a cloned DNA fragment under the specified nonpermissive laboratory conditions designed to represent the natural environment, either by survival of the original host or as a consequences of transmission of the cloned DNA fragment.

Appendix I–I–B–2. For EK2 host-vector systems in which the vector is a phage, no more than one in 10^8 phage particles should be able to perpetuate a cloned DNA fragment under the specified nonpermissive laboratory conditions designed to represent the natural environment either: (i) as a prophage (in the inserted or plasmid form) in the laboratory host used for phage propagation or (ii) by surviving in natural environments and transferring a cloned DNA fragment to other hosts (or their resident prophages).

Appendix I–II—Certification of Host-Vector Systems

Appendix I–II–A. Responsibility. HV1 systems other than *E. coli* K–12 and HV2 host-vector systems may not be designated as such until they have been certified by the Director, NIH. Application for certification of a host-vector system is made by written application to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 3B10, Bethesda, Maryland 20892.

Host-vector systems that are proposed for certification will be reviewed by the RAC (see Section IV–C–1–b–(1)–(e)). This will first involve review of the data on construction, properties, and testing of the proposed host-vector system by a working group composed of one or more members of the RAC and other persons chosen because of their expertise in evaluating such data. The committee will then evaluate the report of the working group and any other available information at a regular review meeting. The Director, NIH, is responsible for certification after receiving the advice of the RAC. Minor modifications of existing certified host-vector systems where the modifications are of minimal or no consequence to the properties relevant to containment may be certified by the Director, NIH, without review by the RAC (see Section IV–C–1–b–(3)–(c)).

When new host-vector systems are certified, notice of the certification will be sent by ORDA to the applicant and to all IBCs and will be published in the *Recombinant DNA Technical Bulletin.* Copies of a list of all currently certified host-vector systems may be obtained from ORDA at any time.

The Director, NIH, may at any time rescind the certification of any host-vector system (see Section IV–C–1–b–(3)–(d)). If certification of a host-vector system is rescinded, NIH will instruct investigators to transfer cloned DNA into a different system or use the clones at a higher physical containment level unless NIH determines that the already constructed clones incorporate adequate biological containment.

Certification of a given system does not extend to modifications of either the host or vector component of that system. Such modified systems must be independently certified by the Director, NIH. If modifications are minor, it may only be necessary for the investigator to submit data showing that the modifications have either improved or not impaired the major phenotypic traits on which the containment of the system depends. Substantial modifications of a certified system require the submission of complete testing data.

Appendix I–II–B. Data to be Submitted for Certification.

Appendix I–II–B–1. HV1 Systems Other than E. coli K–12. The following types of data shall be submitted, modified as appropriate for the particular system under consideration: (i) A description of the organism and vector; the strain's natural habitat and growth requirements; its physiological properties, particularly those related to its reproduction and survival and the mechanisms by which it exchanges genetic information; the range of organisms with which this organism normally exchanges genetic information and what sort of information is exchanged; and any relevant information on its pathogenicity or toxicity; (ii) a description of the history of the particular strains and vectors to be used, including data on any mutations which render this organism less able to survive or transmit genetic information; and (iii) a general description of the range of experiments contemplated with emphasis on the need for developing such an HV1 system.

Appendix I–II–B–2. HV2 Systems. Investigators planning to request HV2 certification for host-vector systems can obtain instructions from ORDA concerning data to be submitted [14–15]. In general, the following types of data are required: (i) Description of construction steps with indication of source, properties, and manner of introduction of genetic traits; (ii) quantitative data on the stability of genetic traits that contribute to the containment of the system; (iii) data on the survival of the host-vector system under nonpermissive laboratory conditions designed to represent the relevant natural environment; (iv) Data on transmissibility of the vector and/or a cloned DNA fragment under both permissive and nonpermissive conditions; (v) data on all other properties of the system which affect containment and utility, including

information on yields of phage or plasmid molecules, ease of DNA isolation, and ease of transfection or transformation; and (vi) in some cases, the investigator may be asked to submit data on survival and vector transmissibility from experiments in which the host-vector is fed to laboratory animals and human subjects. Such *in vivo* data may be required to confirm the validity of predicting *in vivo* survival on the basis of *in vitro* experiments.

Data must be submitted in writing to ORDA. Ten to twelve weeks are normally required for review and circulation of the data prior to the meeting at which such data can be considered by the RAC. Investigators are encouraged to publish their data on the construction, properties, and testing of proposed HV2 systems prior to consideration of the system by the RAC and its subcommittee. More specific instructions concerning the type of data to be submitted to NIH for proposed EK2 systems involving either plasmids or bacteriophage in *E. coli* K–12 are available from ORDA.

Appendix I–III—Footnotes and References of Appendix I

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15. Szybalski, W., A. Skalka, S. Gottesman, A. Campbell, and D. Botstein (1978). *Standardized Laboratory Tests for EK2 Certification.* Gene *3*, 35–38.

Appendix J—Biotechnology Science Coordinating Committee

The following excerpts from its charter (signed October 30, 1985) describe the Biotechnology Science Coordinating Committee:

Purpose

The Domestic Policy Working Group on Biotechnology has determined that in the area of biotechnology with its rapid growth of scientific discovery, scientific issues of interagency concern will arise frequently and need to be communicated among the various agencies involved with reviews of biotechnology applications. The Federal Coordinating Council for Science, Engineering, and Technology (FCCSET) established by 42 U.S.C. 6651 is an interagency science committee chaired by the Director of the Office of Science and Technology Policy with the mission of coordinating science activities affecting more than one agency. Committees may be established under FCCSET for addressing particular science issues. Thus, the Biotechnology Science Coordinating Committee (BSCC) is established to provide formally an opportunity for interagency science policy coordination and guidance and for the exchange of information regarding the scientific aspects of biotechnology applications submitted to federal research and regulatory agencies for approval.

Functions

The BSCC will coordinate interagency review of scientific issues related to the assessments and approval of biotechnology research applications and biotechnology product applications and postmarketing surveillance when they involve the use of recombinant RNA, recombinant DNA, cell fusion or similar techniques.

The BSCC will:

(a) Serve as a coordinating forum for addressing scientific problems, sharing information, and developing consensus;

(b) Promote consistency in the development of Federal agencies' review procedures and assessments;

(c) Facilitate continuing cooperation among Federal agencies on emerging scientific issues; and

(d) Identify gaps in scientific knowledge.

Authority

To accomplish these functions the BSCC is authorized to:

(a) Receive documentation from agencies necessary for the performance of its function;

(b) Conduct analyses of broad scientific issues that extend beyond those of any one agency;

(c) Develop generic scientific recommendations that can be applied to similar, recurring applications;

(d) Convene workshops, symposia, and generic research projects related to scientific issues in biotechnology; and

(e) Hold periodic public meetings.

Members and Chairman

The BSCC includes the following initial members:

Department of Agriculture
- Assistant Secretary for Marketing and Inspection Services
- Assistant Secretary for Science and Education

Department of Health and Human Services
- Commissioner, Food and Drug Administration
- Director, National Institutes of Health

Environmental Protection Agency
- Assistant Administrator for Pesticides and Toxic Substances
- Assistant Administrator for Research and Development

National Science Foundation
- Assistant Director of Biological, Behavorial & Social Sciences

The BSCC is chaired by the Assistant Director for Biological, Behavioral and Social Sciences of the National Science Foundation and the Director of the National Institutes of Health on a rotating basis.

Administrative Provisions

(a) The BSCC will report to the FCCSET through the Chair.

(b) Meetings of the BSCC shall be held periodically. Some public meetings will be held.

(c) Confidential business information and proprietary information shall be protected under the confidentiality requirements of each member agency.

(d) Subcommittees and working groups, with participation not restricted to BSCC members or full-time Federal employees, may be formed to assist the BSCC in its work.

(e) All BSCC members will be full-time Federal employees whose compensation, reimbursement for travel expenses and other costs shall be borne by their respective agencies.

(f) Each member of the BSCC shall provide such agency support and resources as may be available and necessary for the operation of the BSCC including undertaking special studies as come within the functions assigned herein.

(g) An Office of Science and Technology Policy staff member will serve as BSCC Executive Secretary.

Appendix K—Physical Containment for Large-Scale Uses of Organisms Containing Recombinant DNA Molecules

This part of the Guidelines specifices physical containment guidelines for large-scale (greater than 10 liters of culture) research or production involving viable organisms containing recombinant DNA molecules. It shall apply to large-scale research or production activities as specified in Section III–B–5 of the Guidelines.

All provisions of the Guidelines shall apply to large-scale research or production activities with the following modifications:

- Appendix K shall replace Appendix G when quantities in excess of 10 liters of culture are involved in research or production.
- The institutions shall appoint a Biological Safety Officer (BSO) if it engages in large-scale research or production activities involving viable organisms containing recombinant DNA molecules. The duties of the BSO shall include those specified in Section IV–B–4 of the Guidelines.
- The institution shall establish and maintain a health surveillance program for personnel engaged in large-scale research or production activities involving viable organisms containing recombinant DNA molecules which require BL3 containment at the laboratory scale. The program shall include: preassignment and periodic physical and medical examinations; collection, maintenance and analysis of serum specimens for monitoring serologic changes that may result from the employee's work experience; and provisions for the investigation of any serious, unusual or extended illnesses of employees to determine possible occupational origin.

Appendix K–I.—Selection of Physical Containment Levels.

The selection of the physical containment level required for recombinant DNA research or production involving more than 10 liters of culture is based on the containment guidelines established in Part III of the Guidelines. For purposes of large-scale research or production, three physical containment levels are established. These are referred to as BL1–LS, BL2–LS, and BL3–LS. The BL–LS level of physical containment is required for large-scale research or production of viable organisms containing recombinant DNA molecules which require BLI containment at the laboratory scale. (The BL1–LS level of physical containment is recommended for large-scale research or production of viable organisms for which BLI is recommended at the laboratory scale such as those described in Appendix C.) The BL2–LS level of physical containment is required for large-scale research or production of viable organisms containing recombinant DNA molecules which require BL2 containment at the laboratory scale. The BL3–LS level of physical containment is required for large-scale research or production of viable organisms containing recombinant DNA molecules which require BL3 containment at the laboratory scale. No provisions are made for large-scale research or production of viable organisms containing recombinant DNA molecules which require BL4 containment at the laboratory scale. If necessary, these requirements will be established by NIH on an individual basis.

Appendix K–II—BL1–LS Level

Appendix K–II–A. Cultures of viable organisms containing recombinant DNA molecules shall be handled in a closed system (e.g., closed vessel used for the propagation and growth of cultures) or other primary containment equipment (e.g., biological safety cabinet containing a centrifuge used to process culture fluids) which is designed to reduce the potential for escape of viable organisms. Volumes less than 10 liters may be handled outside of a closed system or other primary containment equipment provided all physical containment requirements specified in Appendix G–II–A of the Guidelines are met.

Appendix K–II–B. Culture fluids (except as allowed in Appendix K–II–C) shall not be removed from a closed system or other primary containment equipment unless the viable organisms containing recombinant DNA molecules have been inactivated by a validated inactivation procedure. A validated inactivation procedure is one which has been demonstrated to be effective using the organism that will serve as the host for propagating the recombinant DNA molecules.

Appendix K–II–C. Sample collection from a closed system, the addition of materials to a closed system, and the transfer of culture fluids from one closed system to another shall be done in a manner which minimizes the release of aerosols or contamination of exposed surfaces.

Appendix K–II–D. Exhaust gases removed from a closed system or other primary containment equipment shall be treated by filters which have efficiencies equivalent to HEPA filters or by other equivalent procedures (e.g., incineration) to minimize the release of viable organisms containing recombinant DNA molecules to the environment.

Appendix K–II–E. A closed system or other primary containment equipment that has contained viable organisms containing recombinant DNA molecules shall not be opened for maintenance or other purposes unless it has been sterilized by a validated sterilization procedure. A validated sterilization procedure is one which has been demonstrated to be effective using the organism that will serve as the host for propagating the recombinant DNA molecules.

Appendix K–II–F. Emergency plans required by Section IV–B–3–f shall include methods and procedures for handling large losses of culture on an emergency basis.

Appendix K–III—BL2–LS Level

Appendix K–III–A. Cultures of viable organisms containing recombinant DNA molecules shall be handled in a closed system (e.g., closed vessel used for the propagation and growth of cultures) or other primary containment equipment (e.g., Class III biological safety cabinet containing a centrifuge used to process culture fluids) which is designed to prevent the escape of viable organisms. Volumes less than 10 liters may be handled outside of a closed system or other primary containment equipment provided all physical containment requirements specified in Appendix G–II–B of the Guidelines are met.

Appendix K–III–B. Culture fluids (except as allowed in Appendix K–III–C)

shall not be removed from a closed system or other primary containment equipment unless the viable organisms containing recombinant DNA molecules have been inactivated by a validated inactivation procedure. A validated inactivation procedure is one which has been demonstrated to be effective using the organism that will serve as the host for propagating the recombinant DNA molecules.

Appendix K–III–C. Sample collection from a closed system, the addition of materials to a closed system, and the transfer of cultures fluids from one closed system to another shall be done in a manner which prevents the release of aerosols or contamination of exposed surfaces.

Appendix K–III–D. Exhaust gases removed from a closed system or other primary containment equipment shall be treated by filters which have efficiencies equivalent to HEPA filters or by other equivalent procedures (e.g., incineration) to prevent the release of viable organisms containing recombinant DNA molecules to the environment.

Appendix K–III–E. A closed system or other primary containment equipment that has contained viable organisms containing recombinant DNA molecules shall not be opened for maintenance or other purposes unless it has been sterilized by a validated sterilization procedure. A validated sterilization procedure is one which has been demonstrated to be effective using the organisms that will serve as the host for propagating the recombinant DNA molecules.

Appendix K–III–F. Rotating seals and other mechanical devices directly associated with a closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules shall be designed to prevent leakage or shall be fully enclosed in ventilated housings that are exhausted through filters which have efficiencies equivalent to HEPA filters or through other equivalent treatment devices.

Appendix K–III–G. A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules and other primary containment equipment used to contain operations involving viable organisms containing recombinant DNA molecules shall include monitoring or sensing devices that monitor the integrity of containment during operations.

Appendix K–III–H. A closed system used for the propagation and growth of viable organisms containing the recombinant DNA molecules shall be tested for integrity of the containment features using the organism that will serve as the host for propagating recombinant DNA molecules. Testing shall be accomplished prior to the introduction of viable organisms containing recombinant DNA molecules and following modification or replacement of essential containment features. Procedures and methods used in the testing shall be appropriate for the equipment design and for recovery and demonstration of the test organism. Records of tests and results shall be maintained on file.

Appendix K–III–I. A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules shall be permanently identified. This identification shall be used in all records reflecting testing, operation, and maintenance and in all documentation relating to use of this equipment for research or production activities involving viable organisms containing recombinant DNA molecules.

Appendix K–III–J. The universal biohazard sign shall be posted on each closed system and primary containment equipment when used to contain viable organisms containing recombinant DNA molecules.

Appendix K–III–K. Emergency plans required by Section IV–B–3–f shall include methods and procedures for handling large losses of culture on an emergency basis.

Appendix K–IV—BL2–LS Level

Appendix K–IV–A. Cultures of viable organisms containing recombinant DNA molecules shall be handled in a closed system (e.g., closed vessels used for the propagation and growth of cultures) or other primary containment equipment (e.g., Class III biological safety cabinet containing a centrifuge used to process culture fluids) which is designed to prevent the escape of viable organisms. Volumes less than 10 liters may be handled outside of a closed system provided all physical containment requirements specified in Appendix G–II–C of the Guidelines are met.

Appendix K–IV–B. Culture fluids (except as allowed in Appendix K–IV–C) shall not be removed from a closed system or other primary containment equipment unless the viable organisms containing recombinant DNA molecules have been inactivated by a validated inactivation procedure. A validated inactivation procedure is one which has been demonstrated to be effective using the organisms that will serve as the host for propagating the recombinant DNA molecules.

Appendix K–IV–C. Sample collection from a closed system, the addition of materials to a closed system, and the transfer of culture fluids from one closed system to another shall be done in a manner which prevents the release of aerosols or contamination of exposed surfaces.

Appendix K–IV–D. Exhaust gases removed from a closed system or other primary containment equipment shall be treated by filters which have efficiencies equivalent to HEPA filters or by other equivalent procedures (e.g., incineration) to prevent the release of viable organisms containing recombinant DNA molecules to the environment.

Appendix K–IV–E. A closed system or other primary containment equipment that has contained viable organisms containing recombinant DNA molecules shall not be opened for maintenance or other purposes unless it has been sterilized by a validated sterilization procedure. A validated sterilization procedure is one which has been demonstrated to be effective using the organisms that will serve as the host for propagating the recombinant DNA molecules.

Appendix K–IV–F. A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules shall be operated so that the space above the culture level will be maintained at a pressure as low as possible, consistent with equipment design, in order to maintain the integrity of containment features.

Appendix K–IV–G. Rotating seals and other mechanical devices directly associated with a closed system used to contain viable organisms containing recombinant DNA molecules shall be designed to prevent leakage or shall be fully enclosed in ventilated housings that are exhausted through filters which have efficiencies equivalent to HEPA filters or through other equivalent treatment devices.

Appendix K–IV–H. A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules and other primary containment equipment used to contain operations involving viable organisms containing recombinant DNA molecules shall include monitoring or sensing devices that monitor the integrity of containment during operations.

Appendix K–IV–I. A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules shall be tested for integrity of the containment features using the organisms that will serve as the host for propagating the recombinant DNA molecules. Testing shall be accomplished prior to the

introduction of viable organisms containing recombinant DNA molecules and following modification or replacement of essential containment features. Procedures and methods used in the testing shall be appropriate for the equipment design and for recovery and demonstration of the test organism. Records of tests and results shall be maintained on file.

Appendix K–IV–J. A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules shall be permanently identified. This identification shall be used in all records reflecting testing, operation, and maintenance and in all documentation relating to the use of this equipment for research production activities involving viable organisms containing recombinant DNA molecules.

Appendix K–IV–K. The universal biohazard sign shall be posted on each closed system and primary containment equipment when used to contain viable organisms containing recombinant DNA molecules.

Appendix K–IV–L. Emergency plans required by Section IV–B–3–f shall include methods and procedures for handling large losses of culture on an emergency basis.

Appendix K–IV–M. Closed systems and other primary containment equipment used in handling cultures of viable organisms containing recombinant DNA molecules shall be located within a controlled area which meets the following requirments:

Appendix K–IV–M–1. The controlled area shall have a separate entry area. The entry area shall be a double-doored space such as an air lock, anteroom, or change room that separates the controlled area from the balance of the facility.

Appendix K–IV–M–2. The surfaces of walls, ceilings, and floors in the controlled area shall be such as to permit ready cleaning and decontamination.

Appendix K–IV–M–3. Penetrations into the controlled area shall be sealed to permit liquid or vapor phase space decontamination.

Appendix K–IV–M–4. All utilities and service or process piping and wiring entering the controlled area shall be protected against contamination.

Appendix K–IV–M–5. Hand-washing facilities equipped with foot, elbow, or automatically operated valves shall be located at each major work area and near each primary exit.

Appendix K–IV–M–6. A shower facility shall be provided. This facility shall be located in close proximity to the controlled area.

Appendix K–IV–M–7. The controlled area shall be designed to preclude release of culture fluids outside the controlled area in the event of an accidental spill or release from the closed systems or other primary containment equipment.

Appendix K–IV–M–8. The controlled area shall have a ventilation system that is capable of controlling air movement. The movement of air shall be from areas of lower contamination potential to areas of higher contamination potential. If the ventilation system provides positive pressure supply air, the system shall operate in a manner that prevents the reversal of the direction of air movement or shall be equipped with an alarm that would be actuated in the event that reversal in the direction of air movement were to occur. The exhaust air from the controlled area shall not be recirculated to other areas of the facility. The exhaust air from the controlled area may be discharged to the outdoors without filtration or other means for effectively reducing an accidental aerosol burden provided that it can be dispersed clear or occupied buildings and air intakes.

Appendix K–IV–N. The following personnel and operational practices shall be required:

Appendix K–IV–N–1. Personnel entry into the controlled area shall be through the entry area specifed in Appendix K–IV–M–I.

Appendix K–IV–N–2. Persons entering the controlled area shall exchange or cover their personal clothing with work garments such as jumpsuits, laboratory coats, pants and shirts, head cover, and shoes or shoe covers. On exit from the controlled area the work clothing may be stored in a locker separate from that used for personal clothing or discarded for laundering. Clothing shall be decontaminated before laundering.

Appendix K–IV–N–3. Entry into the controlled area during periods when work is in progress shall be restricted to those persons required to meet program or support needs. Prior to entry all persons shall be informed of the operating practices, emergency procedures, and the nature of the work conducted.

Appendix K–IV–N–4. Persons under 18 years of age shall not be permitted to enter the controlled area.

Appendix K–IV–N–5. The universal biohazard sign shall be posted on entry doors to the controlled area and all internal doors when any work involving the organism is in progress. This includes periods when decontamination procedures are in progress. The sign posted on the entry doors to the controlled area shall include a statement of agents in use and personnel authorized to enter the controlled area.

Appendix K–IV–N–6. The controlled area shall be kept neat and clean.

Appendix K–IV–N–7. Eating, drinking, smoking, and storage of food are prohibited in the controlled area.

Appendix K–IV–N–8. Animals and plants shall be excluded from the controlled area.

Appendix K–IV–N–9. An effective insect and rodent control program shall be maintained.

Appendix K–IV–N–10. Access doors to the controlled area shall be kept closed, except as necessary for access, while work is in progress. Serve doors leading directly outdoors shall be sealed and locked while work is in progress.

Appendix K–IV–N–11. Persons shall wash their hands when leaving the controlled area.

Appendix K–IV–N–12. Persons working in the controlled area shall be trained in emergency procedures.

Appendix K–IV–N–13. Equipment and materials required for the management of accidents involving viable organisms containing recombinant DNA molecules shall be available in the controlled area.

Appendix K–IV–N–14. The controlled area shall be decontaminated in accordance with established procedures following spills or other accidental release of viable organisms containing recombinant DNA molecules.

Appendix L—Release Into the Environment of Certain Plants

Appendix L–I—General Information

Appendix L specifies conditions under which certain plants as specified below, may be approved for release into the environment. Experiments in this category cannot be initiated without submission of relevant information on the proposed experiment to NIH, review by the RAC Plant Working Group, and specific approval by NIH. Such experiments also require the approval of the IBC before initiation. Information on specific experiments which have been approved will be available in ORDA and will be listed in Appendix L–III when the Guidelines are republished.

Experiments which do not meet the specifications of Appendix L–II fall under Section III–A and require RAC review and NIH and IBC approval before initiation.

Appendix L–II—Criteria Allowing Review by the RAC Plant Working Group Without the Requirement for Full RAC Review

Approval may be granted by ORDA in consultation with the Plant Working

Group without the requirement for full RAC review (IBC review is also necessary) for growing plants containing recombinant DNA in the field under the following conditions:

Appendix L–II–A. The plant species is a cultivated crop of a genus that has no species known to be a noxious weed.

Appendix L–II–B. The introduced DNA consists of well-characterized genes containing no sequences harmful to humans, animals, or plants.

Appendix L–II–C. The vector consists of DNA: (i) From exempt host-vector systems (Appendix C); (ii) from plants of the same or closely related species; (iii) from nonpathogenic prokaryotes or nonpathogenic lower eukaryotic plants; (iv) from plant pathogens only if sequences resulting in production of disease symptoms have been deleted; or (v) chimeric vectors constructed from sequences defined in (i) to (iv) above. The DNA may be introduced by any suitable method. If sequences resulting in production of disease symptoms are retained for purposes of introducing the DNA into the plant, greenhouse-grown plants must be shown to be free of such sequences before such plants, derivatives, or seed from them can be used in field tests.

Appendix L–II–D. Plants are grown in controlled access fields under specified conditions appropriate for the plant under study and the geographical location. Such conditions should include provisions for safe, good cultural and pest control practices, for physical isolation from plants of the same species outside of the experimental plot in accordance with pollination characteristics of the species, and for further preventing plants containing recombinant DNA from becoming established in the environment. Review by the IBC should include an appraisal by scientists knowledgeable of the crop, its production practices, and the local geographical conditions. Procedures for assessing alterations in and the spread of organisms containing recombinant DNA must be developed. The results of the outlined tests must be submitted for review by the IBC. Copies must also be submitted to the Plant Working Group of the RAC.

Appendix L–III—Specific Approvals

As of publication of the revised Guidelines, no specific proposals have been approved. An updated list may be obtained from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 3B10, Bethesda, Maryland 20892.

(OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592) requires a statement concerning the official government programs contained in the *Catalog of Federal Domestic Assistance*. Normally NIH lists in its announcements the number and title of affected individual programs for the guidance of the public. Because the guidance in this notice covers not only virtually every NIH program but also essentially every Federal research program in which DNA recombinant molecule techniques could be used, it has been determined to be not cost effective or in the public interest to attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could not be certain that every federal program would be included as many Federal agencies, as well as private organizations, both national and international, have elected to follow the NIH Guidelines. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the *Catalog of Federal Domestic Assistance* are affected.)

Dated: April 18, 1986.

Thomas E. Malone,

Acting Director, National Institutes of Health.

[FR Doc. 86–10120 Filed 5–6–86; 8:45 am]

BILLING CODE 4140–01–M

federal register

Friday
December 19, 1986

Part III

Department of Health and Human Services

National Institutes of Health

Recombinant DNA Research; Advisory Committee Meeting and Proposed Actions Under Guidelines Notices

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes Of Health

Recombinant DNA Advisory Committee; Meeting

Pursuant to Pub. L. 92-463, notice is hereby given of a meeting of the Recombinant DNA Advisory Committee at the National Institutes of Health, Building 1, Wilson Hall, 9000 Rockville Pike, Bethesda, Maryland 20892, on February 2, 1987, from approximately 9 a.m. to adjournment at approximately 5 p.m. This meeting will be open to the public to discuss:

Amendment of Guidelines; and other matters to be considered by the Committee.

Attendance by the public will be limited to space available. Members of the public wishing to speak at the meeting may be given such an opportunity at the discretion of the Chair.

Dr. William J. Gartland, Executive Secretary, Recombinant DNA Advisory Committee, National Institutes of Health, Building 31, Room 3B10, Bethesda, Maryland, telephone (301) 496-6051, will provide materials to be discussed at the meeting, rosters of committee members, and substantive program informaton. A summary of the meeting will be available at a later date.

OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592) requires a statement concerning the official government programs contained in the *Catalog of Federal Domestic Assistance*. Normally NIH lists in its announcements the number and title of affected individual programs for the guidance of the public. Because the guidance in this notice covers not only virtually every NIH program but also essentially every Federal research program in which DNA recombinant molecule techniques could be used, it has been determined to be not cost effective or in the public interest to attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could not be certain that every Federal program would be included as many federal agencies, as well as private organizations, both national and international, have elected to follow the NIH Guidelines. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the *Catalog of Federal Domestic Assistance* are affected.

Dated: December 10, 1986.

Betty J. Beveridge,

Committee Management Officer, NIH.

[FR Doc. 86-28441 Filed 12-8-86; 8:45 am]

BILLING CODE 4140-01-M

Recombinant DNA Research: Proposed Actions Under Guidelines

AGENCY: National Institutes of Health, PHS, DHHS.

ACTION: Notice of proposed actions under NIH guidelines for research involving recombinant DNA molecules.

SUMMARY: This notice sets forth proposed actions to be taken under the National Institutes of Health (NIH) Guidelines for Research Involving Recombinant DNA Molecules. Interested parties are invited to submit comments concerning these proposals. These proposals will be considered by the Recombinant DNA Advisory Committee (RAC) at its meeting on February 2, 1987. After consideration of these proposals and comments by the RAC, the Director of the National Institutes of Health will issue decisions on these proposals in accord with the Guidelines.

DATE: Comments received by January 22, 1987, will be reproduced and distributed to the RAC for consideration at its February 2, 1987, meeting.

ADDRESS: Written comments and recommendations should be submitted to the Director, Office of Recombinant DNA Activities, Building 31, Room 3B10, National Institutes of Health, Bethesda, Maryland 20892. All comments received in timely response to this notice will be considered and will be available for public inspection in the above office on weekdays between the hours of 8:30 a.m. and 5:00 p.m.

FOR FURTHER INFORMATION: Background documentation and additional information can be obtained from the Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland 20892, (301) 496-6051.

SUPPLEMENTARY INFORMATION: The NIH will consider the following actions under the NIH Guidelines for Research Involving Recombinant DNA Molecules.

I. Proposed Amendments of Sections I-A and III-A of the NIH Guidelines

Dr. Bernard Talbot, Deputy Director, National Institute of Allergy and Infectious Diseases, has requested that the following proposed amendments of the NIH Guidelines and rationale be published for comment and considered by the RAC:

The current NIH Guidelines for Research Involving Recombinant DNA Molecules (Guidelines) contain the following text in section III-A of the Guidelines.

> If the experiments in this category are submitted for review to another Federal agency, the submitter shall notify ORDA; ORDA may then determine that such review serves the same purpose, and based on that determination, notify the submitter that no RAC review will take place, no NIH approval is necessary, and the experiment may proceed upon approval from the other Federal agency.

This text appears in section III-A of the Guidelines and is applicable only to experiments covered by Section III-A.

It requires that: (1) An investigator who has submitted a proposal to another Federal agency notify the NIH Office of Recombinant DNA Activities (ORDA); (2) ORDA determine if the review serves the same purpose [as NIH review]; (3) and, if so, ORDA notify the submitter that the experiment may proceed upon approval from the other Federal agency.

On June 26, 1986, the Office of Science and Technology Policy published in the **Federal Register** (51 FR 23302) a "*Coordinated Framework for Regulation* of Biotechnology." It contains a Preamble, followed by Statements of Policy from the Food and Drug Administration, Environmental Protection Agency, U.S. Department of Agriculture, Occupational Safety and Health Administration, and the National Institutes of Health. The Preamble states that,

> . . . for contained federally funded research for biomedical and agricultural purposes, research approval will be granted by the funding agency. . . . Jurisdiction for release may be under S&E, NSF, APHIS, or EPA.

There is no mention in the June 26 **Federal Register** document of any requirement, once approval for a recombinant DNA experiment is obtained from a Federal agency other than NIH, for communication with the NIH Office of Recombinant DNA Activities. And indeed, I believe that the absence of such a requirement should be the case; not only for experiments covered by Section III-A of the Guidelines, but for all recombinant DNA experiments.

Therefore, I propose the following changes in the NIH Guidelines for *Research Involving Recombinant DNA* Molecules.

1. Delete from section III-A of the Guidelines the following paragraph:

> If the experiments in this category are submitted for review to another Federal

agency, the submitter shall notify ORDA; ORDA may then determine that such review serves the same purpose, and based on that determination, notify the submitted that no RAC review will take place, no NIH approval is necessary, and the experiment may proceed upon approval from the other Federal agency.

2. Add at the end of section I-A of the Guidelines the following paragraph:

Any recombinant DNA experiment which according to these Guidelines requires approval by the National Institutes of Health (NIH), may be sent by the submitter to the NIH or to another Federal agency that has jurisdiction for review and approval. Once approval for a recombinant DNA experiment has been given by a Federal agency other than the NIH (whether referred to that agency by the NIH, or sent directly there by the submitter), the experiment may proceed without the necessity for NIH review or approval.

II. Proposed Revision of Section III-A-2 of the NIH Guidelines

Section III-A-2 of the NIH Guidelines currently reads as follows:

III-A-2. Deliberate release into the environment of any organism containing recombinant DNA, except certain plants as described in Appendix L.

At its meeting on September 29, 1986, the RAC voted to recommend that section III-A-2 be revised to read as follows:

III-A-2. Deliberate release into the environment of any organism containing recombinant DNA except:

a. Certain plants as described in Appendix L.

b. Deletion derivatives not otherwise covered by these Guidelines.

c. Organisms covered in exemption III-D-2.

This recommendation has not yet been acted upon by the Director, NIH, and therefore has not yet been incorporated into the NIH Guidelines.

The RAC Working Group on Definitions met on December 5, 1986, and recommended that section III-A-2 be amended to read as follows:

III-A-2. Deliberate release into the environment of any organism containing recombinant DNA except those listed below. The term "deliberate release" is defined as a planned introduction of recombinant DNA-containing microorganisms, plants, or animals into the environment.

a. Introductions conducted under conditions considered to be accepted scientific practices in which there is adequate evidence of biological and/or physical control of the recombinant DNA-containing organisms. The nature of such evidence is described in Appendices L, M, N, and O.

b. Deletion derivatives not otherwise covered by these Guidelines.

c. Organisms covered in exemption III-D-2.

It was the intent of the working group that Appendix L would be the current Appendix L dealing with plants with future changes to be recommended by the RAC. Appendices M, N, and O would be parallel sections, to be written, covering respectively animals, microorganisms other than vaccines, and vaccines.

The minutes of the December 5, 1986, meeting of the working group will be available prior to the February 2, 1986, RAC meeting.

III. Proposed Revision of Section I-B or Section III-A-2 of the NIH Guidelines

The RAC Working Group on Definitions at its meeting on December 5, 1986, passed the following motion with regard to the definition of recombinant DNA:

The working group agreed with the concept that certain types of recombinant DNA experiments which do not involve the introduction of foreign DNA need not be subjected to special regulation as "recombinant DNA." The working group were split as to whether they preferred dealing with this problem by changing the definition of recombinant DNA or by further modifications of other sections of the Guidelines (e.g., those in III-A-2). Therefore, the working group presents the following two options for public comment and RAC consideration:

1. Change definition of recombinant DNA:

"The first paragraph of section I-B would be revised to read as follows (new words in italics):

In the context of these Guidelines, recombinant DNA molecules are defined as either (i) molecules which are constructed outside living cells by joining *foreign* natural or *foreign* synthetic DNA segments to DNA molecules that can replicate in a living cell, or (ii) DNA molecules that result from the replication of those described in (i) above.

The following new footnote would be added at the word "foreign":

Rearrangements involving the introduction of DNA from different organisms or different strains of an organism will be considered recombinant DNA. Deletions, single-base changes and rearrangements within a single genome will not involve the introduction of foreign DNA and therefore would not be considered recombinant DNA.

2. Modify Section III-A-2 to read as follows:

III-A-2. Deliberate release into the environment of any organism containing recombinant DNA except those listed below. The term "deliberate release" is defined as a planned introduction of recombinant DNA-containing microorganisms, plants, or animals into the environment.

a. Introductions conducted under conditions considered to be accepted scientific practices in which there is adequate evidence of biological and/or physical control of the recombinant DNA-containing organisms. The nature of such evidence is described in Appendices L, M, N, and O.

b. Deletion derivatives and single base changes not otherwise covered by the Guidelines.

c. Rearrangements and amplification within a single genome. Rearrangements involving the introduction of DNA from different strains of the same organism would not be covered by this exemption.

The minutes of the December 5, 1986, meeting of the Working Group on Definitions will be available prior to the February 2, 1986, RAC meeting.

IV. Proposed Revisions of Appendices C-II, C-III, and C-IV

Dr. Frank E. Young, Commissioner of Food and Drugs, has submitted the following proposed revisions of Appendices C-II, C-III, and C-IV, and rationale:

On June 26, 1986, a major statement of federal policy, the "Coordinated Framework for Regulation of Biotechnolgy", was published (51 FR 23301-93). We believe that important clarifications of regulatory policy are to be found there, but that some minor changes in the NIH Guidelines are required for consistency and clarity.

As noted on page 23304 of the June 26 document, Appendices C-II, C-III, and C-IV of the NIH Guidelines contain the statement that:

For large-scale (LS) fermentation experiments BL1-LS physical containment conditions are recommended. However, following review by the IBC of appropriate data for a particular host-vector system, some latitude in the application of BL1-LS requirements as outlined in Appendix K-II-A through K-II-F is permitted.

The document continues:

The appropriate large-scale containment requirements for many low-risk [r]DNA derived industrial microorganisms will be no greater than those appropriate for the unmodified parental organisms.

Together, these statements imply that the actions of IBCs should ensure that requirements for physical containment of low-risk microorganisms should be appropriately minimal, i.e., only those that are employed routinely for organisms such as *E. coli K-12, B. subtilis*, or *Saccharomyces cerevisiae*. It should be noted that industrial

fermentation has a long and distinguished history and currently accounts for products valued at more than $2 billion annually (attachment, Tables 1–7). All but a minuscule proportion of this production employs non-pathogenic organisms and is carried out safely under conditions significantly less restrictive than the NIH Guidelines' BL1–LS, which requires that recombinant organisms be handled in a closed system, that culture fluids containing viable organisms not be removed from a closed system, that exhaust gases removed from a closed system be treated by filters equivalent to HEPA filters, etc.

To ensure compliance with the NIH Guidelines, the *E. coli* and *Saccharomyces cerevisiae* production organisms used to manufacture the five DNA-derived pharmaceuticals approved by FDA (human insulin, human growth hormone, two alpha-interferons, and hepatitis B vaccine), are indeed grown under containment conditions at least BL1–LS. This degree of containment is expensive, unwieldy and unnecessary.

Despite the interpretation discussed above of the language in the June 26 document, FDA has received numerous inquiries and requests from academics, industrial representatives, and others who have found the language in the June 26 document and the NIH Guidelines not explicit enough for purposes of strategic planning. Therefore, we propose the following amendment to the NIH Guidelines:

In Appendices C–II, C–III, and C–IV, delete the followinng language:

For these exempt laboratory experiments, BL1 physical containment conditions are recommended.

For large-scale (LS) fermentation experiments BL1–LS physical containment conditions are recommended. However, following review by the IBC of appropriate data for a particular host-vector system, some latitude in the application of BL1–LS requirements as outlined in Appendix K–II–A through K–II–F is permitted.

And substitute:

For these exempt laboratory experiments, the appropriate physical containment conditions need be no greater than those for the host organism unmodified by recombinant DNA techniques.

For large-scale (LS) fermentation experiments, the appropriate physical containment conditions need be no greater than those for the host organism unmodified by recombinant DNA techniques.

Thank you. We hope that this proposal will receive consideration by the RAC at the earliest opportunity.

OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592) requires a statement concerning the official government programs contained in the *Catalog of Federal Domestic Assistance*. Normally NIH lists in its announcements the number and title of affected individual programs for the guidance of the public. Because the guidance in this notice covers not only virtually every NIH program but also essentially every Federal research program in which DNA recombinant molecule techniques could be used, it has been determined to be not cost effective or in the public interest to attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could not be certain that every Federal program would be included as many Federal agencies, as well as private organizations, both national and international, have elected to follow the NIH Guidelines. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual Programs listed in the *Catalog of Federal Domestic Assistance* are affected.

Dated: December 11, 1986.

Bernard Talbot,

Acting Director, National Institute of Allergy and Infectious Diseases.

[FR Doc. 86–28442 Field 12–18–86; 8:45 am]

BILLING CODE 4140–01–M

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COMMENTS RECEIVED ON FEDERAL REGISTER, PART III, DECEMBER 19, 1986

1289 A

COMMENTS ON ITEMS I, II, III, AND IV PUBLISHED IN FEDERAL REGISTER, PART III,

DECEMBER 19, 1986

IBA

Industrial Biotechnology Association

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Washington, D.C. 20006
(202) 857-0244
FAX: (202) 857-0237

Alan R. Goldhammer, Ph.D.
Director of
Technical Affairs

January 22, 1987

Dr. William J. Gartland, Jr.
Executive Secretary
Recombinant DNA Advisory Committee
National Institutes of Health
Building 31, Room 3B10
Bethesda, MD 20892

Re: Proposed Revisions of NIH Guidelines

Dear Dr. Gartland:

These comments on the proposed revisions of the NIH Guidelines for Recombinant DNA Research to be discussed at the February 2 NIH Recombinant DNA Advisory Committee (NIH-RAC) meeting are submitted on behalf of the Industrial Biotechnology Association (IBA). IBA is a trade association of 56 member companies engaged in biotechnology ventures. A current membership roster is attached to this letter.

IBA supports all four of the revisions as set forth in the Federal Register of December 19 (51 FR 45650). These revisions will provide needed clarification in several areas. The new definitions will make the Guidelines less ambiguous in regards to the types of genetically altered organisms that will fall under its purview. Additionally, the role of the various federal regulatory agencies will be explicitly recognized under the changes proposed.

Specific comments to the proposed revisions are given below.

SECTION 1

The changes outlined in this section, proposed by Dr. Bernard Talbot, recognize that various federal regulatory agencies have specific statutory authority to review products created through recombinant DNA technology. Implementation of this change will eliminate the requirement for possible dual reviews when that product is reviewed by the appropriate regulatory agency. IBA believes that the authority of the various regulatory agencies and NIH was set out in the Coordinated Framework for Regulation of Biotechnology (51 FR 23302). This new wording brings the Guidelines into accord with that framework.

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Section II

IBA supports the proposed revision to Section III-A-2 and the development of the associated appendices M,N, and O. This would refine the definition of deliberate release that was acted upon at the NIH-RAC meeting of September 29, 1986. In addition, it goes a step further in establishing criteria for appropriate environmental releases of recombinant organisms.

Having established guidelines will ultimately expedite field experiments with those recombinant organisms where adequate physical and/or biological control can be demonstrated. It is important in the implementation of this proposal to convene working groups with the appropriate scientific expertise to develop appendices M, N, and O.

Section III

IBA supports the full intent of the proposal set forth in this section and we suggest that NIH-RAC select Option 1. It is important to note that there is no difference between those microorganisms created through recombinant DNA technology that are phenotypically the same as might arise naturally or through traditional genetic manipulations such as mutation and selection. Hence, the exemption from the Guidelines of those organisms composed of single base changes, deletions, and rearrangements within a single genome are based on sound scientific principals; their naturally occurring counterparts have caused little concern in the past.

Because Option 1 changes the definition of recombinant DNA at the outset of the Guidelines, IBA believes that this will add more clarity. It will insure that there is no ambiguity as to when an organism is defined as recombinant, regardless whether research on this organism is conducted in a contained or field environment. Option 2 may confuse some individuals because it will be located in a later section that is meant to define "deliberate release".

Section IV

IBA supports the proposed changes in this section that are offered by FDA Commissioner Frank Young. The increasing commercial applications of biotechnology in health care and other fields have necessitated the large scale production of recombinant microorganisms. Virtually all of the research and development work as well as production has involved microbial host-vector constructs that are exempt from the laboratory research guidelines.

The host microorganisms, E. coli K-12, Bacillus subtilis and Saccharomyces cervesiae have been safely used at both laboratory and production-scale levels for many years. Recombinant derivatives of these organisms have been demonstrated to be safe, resulting in the exemption from the Guidelines for certain laboratory uses. The safety consideration of recombinant microorganisms are the same regardless of whether they are being used under laboratory conditions or for the large-scale production of cloned gene products. Biological containment is already inherent in these host-vector constructs.

Under the provisions of Appendices C-II, C-III, and C-IV; IBC's are required to set containment conditions when culture volumes greater then 10 liters are being employed. The NIH Guidelines suggest that where appropriate conditions outlined in Appendix K should be followed. However, a certain flexibility is given to the IBC in the three Appendices under consideration in this proposal. Unfortunately, the present wording is ambiguous and IBC's have been reluctant to interpret the term "some latitude" in a meaningful way. This has complicated strategic planning at those companies where facilities to scale-up production are being designed. As a result, all of the pharmaceuticals approved by FDA are produced at BL1-LS, the most restrictive containment option for the two organisms used as hosts.

Dr. Young's proposal would establish criteria for facility design which IBA believes is entirely appropriate. This would give to the IBC's a continuum of containment options to consider. It is important to remember that those products that are produced by the commercial sector are all regulated by an appropriate federal agency such as EPA or FDA. The product review requires a thorough assessment of the manufacturing process and the regulatory agency must be satisfied that the containment conditions for production are safe and environmentally sound. Historically, these regulatory agencies have looked to the NIH-RAC to provide expert advice on scientific questions about recombinant DNA research. IBA believes that NIH-RAC approval of this specific proposal would be in keeping with that advisorial role.

IBA hopes that these comments are useful to NIH-RAC as they deliberate on these issues.

Sincerely,

Alan Goldhammer

AG:td

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John Jennings, M.D.
VICE PRESIDENT
SCIENCE AND TECHNOLOGY

Pharmaceutical Manufacturers Association

January 21, 1987

Dr. William T. Gartland
Director, Office of Recombinant
DNA Activities
Building 31, Room 3B10
National Institutes of Health
Bethesda, Maryland 20892

Dear Dr. Gartland:

Re: Recombinant DNA Research: Proposed Actions under Guidelines.
Federal Register Notice (Vol. 51, pp. 45650-45652, Doc. 86-28442, December 19, 1986)

The Pharmaceutical Manufacturers Association (PMA) is a voluntary non-profit trade association representing over 100 companies engaged in research on, and the development, manufacturing and marketing of, prescription and ethically promoted drugs, biologicals and in vivo diagnostic products. Increasingly, these therapeutic and diagnostic products are created through biotechnological processes. We are, therefore, vitally interested in how biotechnology is addressed under national science policy and in regulatory decisions that affect research and development of biotechnology-derived products. Accordingly, we welcome this opportunity to comment on proposed changes in NIH recombinant DNA (rDNA) research guidelines.

Overall, the PMA recommends adoption of each of the amendments proposed in Sections I, II, III and IV of the Federal Register notice. Detailed comments concerning individual sections are given below.

Section I

The modifications suggested in this section are appropriate and important clarifications of the regulatory processes for review of proposed experiments and reduce the possibility of unnecessary, duplicative review and/or notification.

Section II

The proposed changes, while relatively minor, are important steps toward defining "deliberate release" and allow exemptions for cases where experience provides adequate evidence of biological and/or physical control of the rDNA containing

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organisms. It is important that preparation and publication for comment of Appendices M, N and O, respectively, be completed quickly.

Section III

Of the two options that are presented, Option 1 is preferred because modification of the definition of rDNA assures that exemption from special rDNA regulation will be applicable throughout the research process and not only in the "deliberate release" portion of the research.

Within Option 1, insertion of the word "foreign" in the first paragraph of Section 1-B of the guidelines is appropriate, as proposed. In the proposed footnote, the phrase "or different strains of an organism" should be deleted in order to avoid confusion with Section III-D of the guidelines. The remainder of the proposed footnote is appropriate as written.

Section IV

The proposed revisions described in this section are highly important clarifications of the guidelines for rDNA research and will provide appropriate consistency of policy and practice throughout the research process. Furthermore, the proposed revisions represent the consensus of both the primary pharmaceutical regulatory agency and the industries that are representative of pharmaceutical research using these techniques. Specific comments relevant to the proposed changes in Section IV are:

1. Prior to proceeding to large-scale studies, an evaluation will already have been completed wherein the particular host-vector and vector-construct system has been demonstrated to present no significant safety issues and is deemed exempt at small scale. Once the safety has been determined for the inserted sequences the appropriate containment at any scale is based on the biology of the host organism.

2. While existing guidelines indicate that "some latitude" in the application of BL1-LS requirement is permitted, of the five pharmaceutical products already on the market, none of the products' sponsors utilized the "some latitude" provision, but used BL1-LS containment in large-scale experiments. This suggests that in actual practice local IBCs are reluctant to take the lead in using the "some latitude" provision. Hence, these IBCs require more specific guidance from the NIH RAC.

3. The pharmaceutical industry has a long and distinguished record in fermentation techniques, and member companies will be submitting documentation of their individual histories in this field. The industrys' experience with E. Coli, B. subtilis and Saccharomyces cerevisiae is not as extensive as it is with some other host organisms, but in the time the

industry has been using these organisms, no untoward safety problems have arisen or been suggested in either small and large scale applications. More specifically, many decades of experience in the brewing industry with Saccharomyces cerevisiae indicate the safety of it as a host. Similar experience, using B. subtilis as a host in the detergent industry, exists. E. Coli K-12 has been used safely in medical research for 60 years and as a recombinant host in small and large-scale pharmaceutical applications for 10 years.

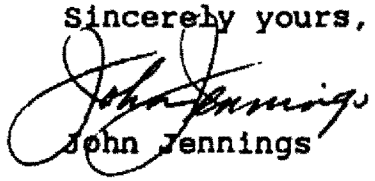
4. We support Dr. Young's proposed revisions in the guidelines and believe that they will enhance the competitive position of the U.S. biotechnology and pharmaceutical industries. We also believe that more explicit guidelines will enhance the strategic planning process at member companies and thus help them compete in the world's market place.

5. As a means to further clarify the language in Appendices C-II, C-III, and C-IV to be consistent with Dr. Young's proposed revisions, we recommend that the following phrase be inserted into the existing second paragraph found on page 45652 uner the paragraph beginning with: And Substitute:

For large-scale [LS] fermentation experiments, and, [insert] where applicable subsequent manufacturing processes, [end insert] the appropriate physical containment conditions need be no greater than those for the host organism unmodified by recombinant DNA techniques.

Lastly, the PMA appreciates the continuing review of the NIH guidelines. Experience has indicated that modifications of the guidelines are appropriate, not only as we gain more experience in the laboratory, but also as we gain more experience at the scale-up stage. PMA member firms are committed to continued voluntary compliance with reasonable guidelines for rDNA research and development.

Sincerely yours,



John Jennings

Genentech, Inc.

January 21, 1987

Dr. William Gartland, Jr.
Executive Secretary
Recombinant DNA Advisory Committee
National Institutes of Allergy
and Infectious Diseases
Building 31, Room 3B10
National Institutes of Health
Bethesda, Maryland 20892

Re: Notice of Proposed Actions under NIH Guidelines
for Research Involving Recombinant DNA Molecules

Dear Dr. Gartland:

Genentech, Inc. is involved in the research, development and manufacture of human pharmaceuticals produced via recombinant DNA technology. As such, we are interested in how commercial rDNA technology is affected by NIH policy and practices; and welcome this opportunity to comment on the proposed changes to the guidelines set forth in the Federal Register, 19 December 1986.

We endorse the four proposed revisions contained in Sections I, II, III and IV. The refined definitions and clarifications will offer Institutional Biosafety Committees clearer guidance in determining appropriate complaince. They will also aid the commercial sector utilizing rDNA techniques in development and manufacture of new products, by establishing appropriate large-scale containment practices based on the knowledge gained through the use of industrial microbes.

Comments on specific proposed actions follow.

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SECTION I

The revisions proposed in this section are appropriately consistant with the regulatory process presented in the published "Coordinated Framework for Regulation of Biotechnology". The proposal makes it clear that duplicative review by Federal agencies is unnecessary.

SECTION II

The proposed revisions provide a useful clarification of what constitutes deliberate release into the environment. However, it is crucial that Appendices M, N and O be prepared by those with appropriate scientific expertise, published for comment, and incorporated into the guidelines in a timely manner.

SECTION III

We agree with the conclusions of the RAC working group that certain types of experiments in which no foreign DNA is introduced into an organism are appropriately not subject to special regulation as "recombinant DNA".

Option 1, to revise the definition of recombinant DNA molecules in Section I-B, is preferred; however, to be consistent with section III-D-3, the phrase "or different strains of an organism" should be deleted.

SECTION IV

Commissioner Young of FDA has proposed changes to the guidelines which we agree are consistent with production-scale practices utilizing safe microorganisms in the pharmaceutical industry.

We have experience utilizing recombinant B. subtilis, S. cerevisiae and E. coli host-vector systems which are exempt from the guidelines for small-scale laboratory uses. Once safety has been determined for the inserted sequences, the appropriate containment at any scale should be based on the biology of the host organism.

In our experience at production scale (e.g. utilizing the recombinant E. coli strain used to produce Protropin), safety characteristics of the host-vector system such as infectivity or pathogenicity have not been changed in the transition from laboratory to manufacturing. We have established a safe record of proceeding from small to large-scale production with a variety of microbial host-vector systems and believe that the proposed change to the guideline reflects this safety-in-use experience.

Physical containment facilities will continue to be designed and operated based on the biological containment features of the production organism. In addition, containment practices and other environmental and occupational health issues relevant to pharmaceutical manufacturing are thoroughly assessed by FDA as part of their regulatory responsibility.

We hope these comments are helpful as NIH-RAC considers the proposed changes, and we are pleased to see the continuing review of the guidelines based on the growing experience with recombinant DNA technology.

Sincerely,

CAROL LAX HOERNER, Ph.D.
Manager, Environmental Health
Biosafety Officer

CLH:smd

4/48

ANIMAL HEALTH INSTITUTE

January 21, 1987

Director, Office of Recombinant DNA Activities
National Institutes of Health
Building 31, Room 3B10
9000 Rockville Pike
Bethesda, MD 20892

RE: Notice of proposed actions under NIH guidelines for research involving recombinant DNA molecules, 51 Fed.Reg. 45650 (December 19, 1986)

Dear Director:

The Animal Health Institute is composed of the major U.S. manufacturers of animal health products. We are pleased to have the opportunity to comment on the changes proposed in the NIH Guidelines, which will have direct or indirect effects on our members engaged in research and development involving recombinant DNA molecules.

We are in full agreement with the changes proposed in Section I, "Proposed Amendments of Sections I-A and III-A of the NIH Guidelines," and Section IV," Proposed Revisions of Appendices C-II, C-III, and C-IV." The changes are justified for the reasons stated in the notice, and we recommend their prompt adoption.

Section II, "Proposed Revision of Section III-A-2 of the NIH Guidelines," offers us a choice. We recommend adoption of the approach of the RAC Working Group on Definitions. We particularly support this approach because of the planned development of Appendix O, which would apparently provide more specific guidance on "deliberate release" of recombinant DNA-containing organisms in connection with use of a vaccine.

With regard to Section III of the notice, "Proposed Revision of Section I-B or Section III-A-2 of the NIH Guidelines," we have no preference between the options, bearing in mind our recommendation regarding vaccines described above.

We thank you for the opportunity to provide these comments, and we congratulate you on the good effort.

Sincerely yours,

Fred H. Holt

Fred H. Holt
President

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FHH:dbk

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Lilly

Lilly Research Laboratories
A Division of Eli Lilly and Company
Lilly Corporate Center
Indianapolis, Indiana 46285

Irving S. Johnson, Ph.D.
Vice President
(317) 276-4391

January 20, 1987

Dr. William J. Gartland, Jr.
Director, Office of Recombinant
DNA Activities
National Institute of Allergy
and Infectious Diseases, 31/3B10
National Institutes of Health
Bethesda, Maryland 20205

Dear Dr. Gartland:

Eli Lilly and Company is a research-based corporation that develops, manufactures, and markets human medicines, medical instrument systems, diagnostic products, agricultural products, and cosmetics. We are actively involved in recombinant DNA research in several areas of life sciences. Therefore, we would like to make the following comments on the proposed actions published in the December 19, 1986, Federal Register, 51, No. 244.

I. Dr. Talbott proposes amendments to Sections I-A and III-A of the NIH Guidelines to relieve the need for the NIH Recombinant Advisory Committee (RAC) to review experiments submitted to other federal agencies with notification of ORDA of the action taken.

We support adoption of the proposed revisions.

II. The Working Group on Definitions of the RAC proposes definitions of deliberate release by revisions of Section III-A-2 of the NIH Guidelines.

We support adoption of the proposed revision of this section which adds clarity to the Guidelines and properly addresses the issue of deliberate release.

III. The Working Group on Definitions of the RAC proposes two alternatives for the definition of recombinant DNA by revision of Section I-B or Section III-A-2 of the NIH Guidelines.

We support adoption of the proposal to clearly redefine recombinant DNA by revision of Section I-B. The concept that certain types of recombinant DNA experiments which do not involve the introduction of foreign DNA need not be subject to special regulation is an extremely important concept. Modification of the definition in Section I-B to define this concept insures that exemptions from special rDNA regulations will be applied throughout the research process and not only in the deliberate release phase.

However, we would propose changes in the wording of the footnote to clarify what we perceive as ambiguities caused by the use of the words "organism" and "strain." It is unclear whether "organism" as presented in this context refers to organism at the genus or species level. The use of the word "strain" in this section is more restricting than current guidelines and creates confusion as it relates to Section III-D (Experiments which are exempt from Guidelines) of the Guidelines. In an attempt to clarify these ambiguities we would propose the following wording for the footnote:

> Rearrangements involving the introduction of DNA from different species of an organism will be considered recombinant DNA, deletions, single-base changes and rearrangements within a single genome will not involve the introduction of foreign DNA and therefore would not be considered recombinant DNA.

In the event that the RAC would choose to redefine recombinant DNA by revision of Section III-A-2 of the Guidelines, we would offer the same justification for changing the wording of the proposal (Section III-A-2-C) from "different strains of the same organism" to "different species of the same organism."

IV. Dr. Frank Young proposes revisions of Appendices C-II, C-III, and C-IV which would permit the large-scale fermentation of E. coli K-12, B. subtilis, or S. cerevisiae if modified by recombinant DNA techniques under the same levels of physical containment as the unmodified organism.

We support the proposed revisions which are significant changes that provide appropriate consistency throughout the

research process. Specific comments regarding the proposed changes are:

A. Industrial experience from decades (E. coli and B. subtilis) to hundreds of years (S. cerevisiae) supports the contention that fermentations using nonrecombinant strains of E. coli, B. subtilis and S. cerevisiae are essentially benign. L-asparaginase, produced by Eli Lilly and Company in the early 1970s, was among the first examples of a commercially available E. coli fermentation product to be used clinically. (Grinnan, E. L., L-Asparaginase: A Case Study of an E. coli Fermentation Product, In: Insulins, Growth Hormone, and Recombinant DNA Technology, John L. Gueriguian, ed., Raven Press, New York, 1981). L-asparaginase was produced in conventional fermenters at the 40,000L scale with no untoward safety problems either to workers or to the environment. Furthermore, we have a long and distinguished record in fermentation techniques which utilize large-scale production of a wide range of organisms, most notably Streptomyces, Actinomyces, Penicillium, and Cephalosporium. Over the last forty years, with the exception of isolated cases of hypersensitivity reactions, we have experienced no health associated risks involving large-scale production processes with these organisms. These reactions when they occurred were always associated with the product from the fermentations rather than any inherent problem associated with the organism itself. If this proposed revision is approved by the NIH Recombinant Advisory Committee and accepted by the Director, NIAID, it would be the policy of this company to immediately report any novel and unexpected health or environmental problem which could be a result of this proposed revision, to the NIAID and the local IBC, as well as the steps taken to address the problem.

B. Risk assessment studies have consistently failed to show any significant risk associated with any of the above-mentioned hosts carrying plasmids coding for peptides of animal or human origin.

C. As was made clear at the Asilomar Conference, the 10L volume limit stipulated in the laboratory Guidelines was one merely of convenience and was not intended to imply that large volumes are significantly more hazardous than

small volumes (most participants used or had access to standard 10L laboratory fermenters).

D. As expressed in the current Guidelines, the IBC has the responsibility for setting containment requirements for large-scale fermentations using exempt microorganisms. Most IBCs, including ours, look to the Guidelines for guidance on these issues. The explicit proposed wording is most helpful in providing that guidance.

We appreciate the opportunity to comment on these proposed changes to the Guidelines. Experience has indicated that as more scientific information accumulates and more experience is gained, such modifications in the Guidelines are appropriate.

Sincerely yours,

Irving S. Johnson

ISJ/rl

GENENCOR

Genencor, Inc. 180 Kimball Way Telephone 415-588-3475
South San Francisco, CA 94080

January 19, 1987

Dr. William J. Gartland, Jr.
Office of Recombinant
DNA Activities
National Institutes of Health
Bethesda, MD 20892

RE: Proposed Actions Under Guidelines(51 FR 45650)

Dear Dr. Gartland:

Genencor, Inc., an enzyme manufacturing company which utilizes rDNA technology and voluntarily complies with the NIH Guidelines for Research Involving Recombinant DNA Molecules, is writting in support of all four proposed revisions to the Guidelines as described in the Federal Register of December 19, 1986 (51 FR 45650).

I. Proposed Amendments of Sections I-A and III-A of the NIH Guidelines

The revisions proposed in this section acknowledge that various regulatory agencies have specific statutory authority to review products created through recombinant DNA technology and can review experiments which might otherwise be reviewed by NIH. Implementation of this change will eliminate the potential of dual review by NIH and the responsible regulatory agency and will implement the statement in the Preamble of the "Coordinated Framework for Regulation of Biotechnology" regarding research approvals. Incorporating the proposed change into the Guidelines will remove any questions concerning review authority.

II. Proposed Revisions of Section III-A-2 of the NIH Guidelines

Genencor supports the proposed revisions to Section III-A-2 and the development of the associated appendices M, N, and O. The proposed definition, when implemented, will provide needed clarity for the definition and exclude certain organisms when used under adequate, defined biogical and/or physical controls. This will eliminate unnecessary oversight for organisms meeting the criteria outlined in Appendices L, M, N and O. We urge the NIH-RAC to approve this proposed revision and to form working groups with the appropriate scientific expertise to develop appendices M, N and O so that the proposals can be implemented.

III. Proposed Revisions of Section I-B or Section III-A-2 of the NIH Guidelines

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While Genencor supports the intent of the proposed changes set forth in options 1. and 2., we encourage the NIH-RAC to adopt option 1.

Option 1 would define recombinant DNA at the beginning of the Guidelines and ensure that there is no ambiguity as to when an organism is defined as recombinant. Option 2, on the other hand, may lead to ambiguity as it endeavors to incorporate the definition of rDNA with the definition of deliberate release.

The exemption of organisms created through single base changes, deletions, and rearrangements within a single genome is based on sound scientific principles and will lead to consistent treatment of such organisms created through rDNA technology and naturally occurring organisms or those derived through traditional genetic manipulations such as mutation and selection.

IV. Proposed Revisions of Appendices C-II, C-III, and C-IV

Genencor supports the changes proposed under this section. As Dr. Young stated, the host organisms in these three Appendices, E. coli K-12, B. subtilis and Saccharaomyces cerevisiae, have been used safely at both the laboratory and production scale for many years. Their exemption from the Guidelines as host-vector systems is further acknowledgment that they are considered safe for recombinant DNA work as well. The "Coordinated Framework for Regulation of Biotechnology" stated that "The appropriate large-scale containment requirements for many low-risk [r]DNA derived industrial microorganisms will be no greater than those appropriate for the unmodified parental organisms." Dr. Young's proposal incorporates the long history of safe use of these organisms as well as the statement in the Coordinated Framework.

Incorporation of the changes proposed by Dr. Young will eliminate any prior ambiguity in the Guidelines and make it clear to IBC's that it is accepted practice to handle these organisms at less than BL1-LS containment. As Dr. Young indicated, without this clarity, IBC's have been reluctant to reduce containment requirements, resulting in levels of containment that are needlessly expensive, unwieldy and unnecessary.

Genencor wishes to thank the NIH-RAC for the opportunity to comment on these proposals and hopes that our comments are useful to you in the decision making process.

Sincerely,

Alice J. Caddow

Alice J. Caddow
Director of Regulatory
and Environmental Affairs

1900 Oak Terrace Lane/Thousand Oaks, California 91320/Telephone 805 499-5725
ITT Telex #4994440 Telecopier 805 499-9315

Ralph Smalling
Regulatory Affairs Specialist

January 19, 1987

Director, Office of Recombinant DNA Activities
Building 31, Room 3B10
National Institutes of Health
Bethesda, MD 20892

Ladies/Gentlemen,

Amgen, a California-based biotechnology company, wishes to comment on the proposals to be considered by the Recombinant DNA Advisory Committee (RAC), as outlined in The Federal Register, Vol. 51, No. 244, dated Friday, December 19, 1986.

We believe these proposals are progressive steps in the rational oversight of experiments using recombinant DNA technology. The proposals seem to us to reflect the scientific data which has been, and continues to be, gathered in this field. Amgen agrees with the concept that experiments involving deletions, single-base changes and rearrangements within a single genome (work in which no foreign DNA is inserted) need not be subjected to special regulation as recombinant DNA experiments. In addition, initiation of experiments involving r-DNA technology following approval by the federal agency with appropriate jurisdiction, without the need for NIH approval, should eliminate unnecessary delay and duplication of effort. It is hoped that the new BSCC framework will insure a consistent approach to such agency reviews.

Amgen agrees that the requirement for BL1-LS containment for the production of r-DNA derived products from low-risk microorganisms is expensive, unwieldy, and unnecessary. We support the proposal that large-scale (LS) fermentation physical containment conditions need be no greater than those for the host organism unmodified by r-DNA techniques. We hope the NIH viewpoint concerning BL1-LS conditions will be extended to other governmental agencies with authority for reviewing manufacturing applications. Such a position is consistent with the long and distinguished history of U.S. industrial fermentation, and the recognition that BL1-LS conditions are unnecessary for the manufacture of the five DNA-derived pharmaceuticals currently approved by FDA.

Please include this letter as a part of the written comments and recommendations to FR Docket 86-28442. Thank you.

Sincerely,

Ralph Smalling

RJS/jdh
0004-0187A

1289 B

COMMENTS ON ITEMS I, II, AND IV PUBLISHED IN FEDERAL REGISTER, PART III,

DECEMBER 19, 1986

CENTRAL RESEARCH
PFIZER INC., EASTERN POINT ROAD, GROTON,CONNECTICUT 06340
203-441-4541

RICHARD L. HINMAN, Ph.D.
Senior Vice President
Chemical Products Research and Development

January 21, 1987

The Director
Office of Recombinant DNA Activities
Building 31, Room 3B10
National Institutes of Health
Bethesda, MD 20892

Dear Sir:

Re: 51 FR 45650-52. Notice of Proposed Actions under NIH Guidelines for Research Involving Recombinant DNA Molecules - Amendments to Sections I-A, III-A, III-A-2, and Appendices C-II, C-III and C-IV

We welcome the opportunity to comment on the above FR proposal and would like to go on record in support of the amendments to Sections I-A, III-A, III-A-2, and Appendices C-II, C-III and C-IV. Furthermore, we would like to offer additional comments in support of the proposal of the Commissioner of Food and Drugs (Dr. Frank R. Young) to amend the subject appendices to the NIH Guidelines.

The NIH Guidelines of June 26, 1986, point out that large-scale containment requirements for many low-risk R.DNA derived industrial microorganisms will be no greater than those for the parent organisms. Dr. Young's proposed revision would explicitly state this principle in the Guidelines. In view of the industry's exemplary safety record in handling the parent organisms, we endorse the Commissioner's proposal.

The fermentation industry has a long and distinguished history of safe operation of processes involving Bacillus subtilis, Escherichia coli K-12 and Saccharomyces cerevisiae at manufacturing scale. Pfizer's incident-free experience with Bacillus subtilis used in the production of detergent enzymes on a worldwide basis for many years is a part of this history of safe commercial operation.

We believe that the industry in general and Pfizer Inc. in particular has demonstrated an exemplary record of safety in handling these organisms through methods which are soundly based on good engineering principles of design and practice. We believe that the requirement of containment of the exempted organisms identified above at the BL1-LS level during large-scale cultivation is unwarranted based on the industry's extensive experience and health and safety record.

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We agree that amendment of the language of the NIH Guideline Appendices as proposed by the Commissioner would serve to ameliorate the cost of implementing unwieldy and unnecessary containment measures by industry. Such action would not, in our opinion, lead to decreased safety margins for employees of corporations engaged in fermentation production of recombinant molecules or lead to increased risk to public health and welfare.

Moreover, relief from unnecessary costs of meeting BL1-LS compliance at large scale could help to increase the industry's international competitiveness in the rapidly-advancing area of recombinant DNA production technology.

Accordingly, we support the proposed substitution of the language recited in 51 FR 45652 for Sections I-A, III-A, III-A-2, and Appendices C-II, C-III and C-IV of the NIH Guidelines for Research Involving Recombinant DNA Molecules.

Sincerely,

Richard L. Hinman

Richard L. Hinman

1289 C

COMMENTS ON ITEMS II and III PUBLISHED IN FEDERAL REGISTER, PART III,

DECEMBER 19, 1986

UNIVERSITY OF WASHINGTON
SEATTLE, WASHINGTON 98195

20 January 1987

Recombinant DNA Advisory Committee
ORDA
NIAID
National Institutes of Health
Bldg. 31, Room 3B-10
Bethesda, MD 20205

Dear RAC Members:

I wish to comment on several aspects of the proposals in the Federal Register for December 19, 1986 which you will be discussing at your February 2, 1987 meeting. As a threshold issue, let me express concern that your Committee and its subgroups appear to be getting a very skewed range of input on their proposals and work. The procedures of the Federal bureaucracy are such that the Register reaches most people only a few days before comments are due, a process which largely precludes reflective commentary. Only if one is on an "inside track" will it be generally possible to provide effective input, and that requires a presence in D.C. and/or lobbyists or paid staffers to monitor meetings, etc.

In other words, your current procedures do not facilitate the receipt of a balanced range of views, and instead favor the over-amplification of the positions of private interests with means and of the bureaucracy itself. For example, none of the non-member attendees at the December 5 meeting of the Working Group on Definitions represented public interest groups. Do you honestly believe that no environmental organizations, to give but one example, have any interest in deliberate release? I urge you to promptly devote some attention to improving your outreach activities and assuring that interested persons have, in fact, enough time to respond to your proposals so that publication in the Federal Register is not just a sham legal formality.

I was mailed the minutes of the December 5th meeting in response to a phone call several weeks ago to obtain information for constructing meaningful comments. These arrived on January 13th with a cover note from an ORDA staffer requiring comments to be received in Bethesda on January 14 if they were to be available for "prior review" by RAC!

For persons who do not share your level of intimate knowledge of the Guidelines, the Federal Register notice regarding deliberate release is somewhat incomplete. Although the current language of III-A-2 is quoted on page 45651, the context within which it exists is not given, and neither grammatically nor logically can it stand alone. Is it an exception to a general rule? Is it a definition? etc. In other words, do the changes proposed (topics II and III of the notice) expand or contract the possibilities or ease of environmental release? While the material can certainly presume that readers have a secondary education, providing context is at least courteous and, indeed, may be essential for comprehensibility.

Substantively, I wish to address the guidelines relevant to gene deletions. I oppose relaxing the Guidelines on this point, as would occur under the definitions of "deliberate release" and/or "recombinant DNA" in topics II and III of the Register notice. My reasons are several:

- o No experimental evidence is cited in support of the proposal and even if the deletion of a gene in one species has only benign consequences this certainly is not scientific proof that the deletion of any other gene in that species, or any gene in any other species, would also be benign.

- o The proposal seems to be bottomed on logic instead of empiricism, and such logic could well be misleading and faulty although apparently straightfoward and simple (see my article "Institutional Biosafety Committees and the Inadequacies of Risk Regulation," Science, Technology and Human Values, Vol. 9, Issue 4, No. 49, Fall 1984, pp. 16-34.) Simplistic syllogisms, such as are behind this deletion proposal, are not always valid. The reasoning seems to be

  A is harmless or of known harm
  B constituent is harmless
  Therefore, A-B is no more harmful.

  Yet if A is a moderate solution of lye (sodium hydroxide) and B is water, then the conclusion is false. If the syllogism need not hold for inanimate substances, how can we rely on it for living material with all its additional vagaries and possibilities of interaction.

- o The deletion of a gene would appear likely to result in the elimination of the production of any proteins that gene codes for, but do we know it will have no effect on the coding sequences of other, perhaps adjacent, genes? I do not believe enough is known about intracellular interactions to reach a conclusion with certainty.

- o The deletion of a gene, and any proteins it helps to produce or regulate, could have substantial ecological effects by altering the organism's pool of available protein substances and thus,

perhaps, its ability to compete for ecological niches; the organism might be afforded an advantage (it extends its realm) or a disadvantage (its range contracts) and this alteration of the environmental balance of organisms could have deleterious effects for human beings (e.g. reducing the occurrance of an economically important species) or for ecological well-being itself.

Therefore, I urge you not to relax the Guidelines regarding gene deletions. The proponents of such a change do not seem to have satisfied a reasonable burden of proof to justify it.

Very truly yours,

Philip L. Bereano
Associate Professor

1289 D

COMMENTS ON ITEMS II, III, AND IV PUBLISHED IN FEDERAL REGISTER, PART III,

DECEMBER 19, 1986

186A South Street
Boston, MA 02111
(617) 423-0650

January 20, 1987

COMMITTEE FOR RESPONSIBLE GENETICS

103

William A. Gartland
Recombinant DNA Advisory Committee
Building 31, Room 3B10
National Institutes of Health
Bethesda, Maryland 20892

Dear Dr. Gartland:

On behalf of the Committee for Responsible Genetics (CRG), I would like to submit the following comments to the Federal Register notice of December 19. We will focus our comments primarily on items II and III, the proposed revision of Section III-A-2 of the NIH Guidelines and item IV, the proposed revisions of Appendices of C-II, C-III, and C-IV.

1) The CRG supports leaving unchanged the definition of recombinant DNA and recommends citing each exemption to the definition within the guidelines. We do not see sufficient empirical justification within the scientific disciplines of the intrinsic safety of deletion mutants of microorganisms to warrant broad exemptions of these products of recombinant DNA from review by RAC. As an example of this, we refer the RAC to the comments of Robert Colwell et al. concerning the Coordinated Framework for Regulation of Biotechnology submitted to the Office of Science and Technology Policy concerning the Federal Register notice of June 26, 1986 for a concise review of many of the questions raised within the scientific community. Dr. Colwell and his colleagues point out that:

> ... Because regulatory regions in the genome serve to control the level of production of gene products, in some cases turning production on or off entirely, ecologically important aspects of phenotype, such as substrate utilization, can certainly be altered by changes in regulatory sequence In the same vein, deletion of regulatory sequences (e.g. the removal of a repressor, or of a promoter) clearly can also control gene expression; the deletion of an entire gene certainly does...However "precisely constructed" an organism may be genetically, its ecological phenotype is not so easily predicted, and is nonetheless a matter for discovery and testing by careful experiments

2) Referring to proposed changes on section III-A-2 c of the guidelines, the CRG objects to the use of the criterion for exemption of laboratory

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experiments as sufficient reasoning to exempt those same organisms for deliberate release. The scale and concentration of organisms involved in an environmental release, in conjunction with the complexity of ecological systems, makes the situations of laboratory and of landscape distinctive. One obvious difference is competition. In a laboratory setting one is not necessarily concerned about competition in the ecosystem, such as the displacement of INA^+ with INA^-. We therefore oppose the proposed change that would exempt organisms from RAC review based solely on this criterion.

3) In reference to the proposed revisions of Appendices C-II, C-III and C-IV, the CRG strongly opposes lessening the BL1-LS physical containment conditions in the NIH guidelines for large-scale fermentation experiments. This action represents a fundamental change in the NIH guidelines and would be a major action for the RAC. The rationale for this position suggests that BL1-LS containment presents an obstacle to commercial development. The CRG does not accept this reasoning as the basis for changing a major policy.

The proposal also neglects to take into account the implications for worker, as well as community, health and safety and what the basis for this exemption should be. At the very minimum the CRG recommends, 1) that this proposal be reviewed by NIOSH and OSHA before implementation and 2) that conclusive evidence be presented for public comment that the removal of the requirement for closed system large scale manufacturing using recombinant organisms will not have a negative impact on the health and safety of the workers in the plant, or on the communities surrounding these plants.

Thank you for considering these comments.

Sincerely,

Nachama L. Wilker
Executive Director

1289 E

COMMENTS ON ITEM III PUBLISHED IN FEDERAL REGISTER, PART III, DECEMBER 19, 1986

THE PUBLIC HEALTH RESEARCH INSTITUTE
OF THE CITY OF NEW YORK, INC.

455 FIRST AVENUE, NEW YORK, N. Y. 10016
TEL. (212) 578-0800

Office of the Director

January 16, 1987

Dr. William S. Gartland
Office of Recombinant DNA Activities
Building 31, Room 3310
National Institutes of Health
Bethesda, MD. 20892

Dear Bill,

I am writing to support Option 1 of the proposal to amend the Guidelines as stated in FR 51, p. 45651, December 19, 1986.

I have previously submitted materials in support of this concept and I enclose herewith two documents. First is a proposal to amend the guidelines essentially as in Option 1, that I prepared last year to be submitted on behalf of the five members of the original "Plasmid Working Group" (PWG) who drafted the document that became the actual basis of the guidelines. This proposal was approved by all except Stan Cohen who had some reservations that were never addressed; consequently, the proposal was never submitted. The second document is the text of a piece I had written at the time in hope of publication in the New York Times. It never got published but I submit it herewith as a more elaborate statement of the same position. It contains diagrams which may be informative to the lay members of RAC.

I believe these documents state fairly clearly my support for Option 1. I know Jon King and Liebe Cavalieri have argued that deletions constructed in vitro by splicing techniques are not equivalent to natural deletion. This argument is not supportable on genetic grounds -- the relevant property of a deletion is that it does not revert; while it is true that the precise endpoints of an in vitro deletion are unlikely to be the same as any natural deletion, I do not see how this fact could have any possible biological consequences per se, or could possibly impact on the biohazard question, or could possibly be used to defend the position that in vitro deletions are in principle different from in vivo ones -- after all, it is only unlikely, not impossible that the two could be identical.

Sincerely yours,

Richard Novick, M.D.

enc.
RN/de

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THE PUBLIC HEALTH RESEARCH INSTITUTE
OF THE CITY OF NEW YORK, INC.

455 FIRST AVENUE, NEW YORK, N. Y. 10016
TEL. (212) 578-0800

May 6, 1986

MEMORANDUM

TO: Recombinant DNA Committee

FROM: R. Novick
R. Clowes
S. Cohen
R. Curtiss III
S. Falkow

RE: Amendment to Guidelines

In view of the recent success enjoyed by Jeremy Rifkin and the Foundation on Economic Trends in blocking the release of ice-crystal mutants of Pseudomonas and the testing of a pseudorabies vaccine, we should like to propose a re-affirmation of the basic philosophy of the Guidelines in the form of an amendment.

When preparing our draft proposal for Asilomar we considered organisms containing material from two or more species as novel and therefore conceivably hazardous. The entire proposal and, we believe, the guidelines themselves, were based entirely upon this concept.

Subsequent scientific progress has resulted in the ability to eliminate any specific gene in a microorganism by cloning, in vitro deletion, and subsequent recombinational replacement. This results in a local deletion and the organism is not in any sense recombinant; indeed, this technology is merely a more sophisticated, more precise, and infinitely more reliable means of accomplishing what plant and animal breeders have been doing for several millenia and what geneticists have been doing for the better part of a century.

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Somehow, the critical distinction between this method of mutation induction and the creation of truly novel recombinant organisms by gene splicing has never been made and therefore the regulatory superstructure that has grown up around recombinant DNA has automatically included both.

It is this scientifically invalid and retrogressive situation that has spawned the opportunistic litigation of Rifkin et al and it needs to be corrected on legal as well as on philosophical grounds.

It is proposed that paragraph I-B of the guidelines be amended to read: "..... (i) molecules which are constructed outside living cells by joining synthetic DNA segments or DNA segments from one or more foreign species to DNA molecules that can replicate ....." The new language is underlined and, as you will perceive, it totally excludes all self-cloning from the Guidelines. While this exclusion is broader than that of just deletions, we feel that it represents an absolutely logical division, based on the above argument; although the objection will be raised that it excludes such experiments as cloning a toxin gene on a high copy plasmid, etc., we would argue (a) that one can easily generate hyper-producing strains without gene splicing and (b) since many toxin genes are transposable, their attachment to a high copy plasmid, specifically, could also occur by natural means.

different

) May 15, 1986

When is a spliced gene not spliced?

In recent months, Jeremy Rifkin and the Foundation on Economic Trends have scandalized the scientific community and the fledgling biotechnology industry by obtaining legal injunctions against the continued testing and projected use of two mutant microorganisms developed with the aid of gene splicing.

The two engineered microorganisms are a strain of Pseudomonas bacteria that can no longer produce a substance around which plant-damaging ice crystals form and an avirulent derivative of pseudorabies virus for use as a vaccine. These two strains have potentially major economic benefits, promising to alleviate frost damage to certain crop plants and to control pseudorabies, a very serious disease of swine. They are thus among the exciting first fruits of modern biotechnology. Although neither of the new strains contains foreign DNA or any spliced gene, the mutant organisms are technically covered by the NIH Guidelines for Recombinant DNA Research, merely because gene splicing techniques were utilized in their development. Consequently, because the laboratories developing and testing the new strains may not have adhered precisely to the extant regulations, based on the Guidelines, that govern the release of gene-spliced organisms into the environment, they left themselves vulnerable to litigation. Admittedly, the legal decisions in both cases were technically correct; but the true basis of this unfortunate sequence of events is a scientifically invalid provision of the Guidelines that has carried over into legally binding regulations.

As chairman of the group of five scientists who prepared a document that served as the first draft of the guidelines in 1974, I can state with some assurance that our purpose was to ensure that novel hybrid organisms produced by the splicing in the test tube of genes from two or more progenitor species would

be handled with care because of the possibility that they might have unpredictable, harmful biological properties. A potentially hazardous experiment that was commonly cited as an example at the time is the construction of hybrid E. coli bacteria able to produce diphtheria toxin. Appropriately, biological studies of diphtheria toxin cloned in E. coli are performed at the highest available level of containment to avoid any possibility of accidental release; safety precautions are also appropriate during testing of recently developed hybrid vaccinia viruses potentially useful as vaccines against AIDS, herpes, etc., to ensure that these virues are not inadvertently released before their safety has been adequately assessed.

The Pseudomonas and pseudorabies strains, however, do not pose the same safety issue precisely because they were not produced by joining genes from two or more species; instead, they represent an entirely different application of gene splicing, namely a precise, accurate, and virtually fail-safe method of eliminating a single specific gene from any microorganism. In this method, the unwanted gene is first cloned into a laboratory strain of E. coli, where it can be conveniently manipulated. An essential segment of the gene is then snipped out (deleted) and the now inactive gene is returned to its parent organism where a natural recombination process inserts the defective gene in place of the native, active one. The net effect is the precise removal of an essential part of the unwanted gene; no foreign genetic material is involved. The power of gene splicing technology in this case is that it permits the isolation, amplification and manipulation of the gene in the test tube. All of the test tube steps are, of course, performed in accordance with the Guidelines, since these do involve gene splicing. This type of genetic manipulation is, basically, nothing but a more sophisticated method of accomplishing what plant

and animal breeders have been doing for several millenia and what geneticists have been doing for over a century, namely selecting or creating mutations that alter a specific genetic trait, either for practical or for experimental purposes. There has not been, nor should there be, any type of regulation of these older types of experiments since they involve simply the utilization of entirely natural processes.

The use of gene-splicing methods for permanently and precisely inactivating specific genes was simply not foreseen in the early 70's when the Guidelines were written; indeed, the critical distinction between this type of gene splicing and that involving the creation of hybrid organisms containing genetic material from two or more different species has never been made. Had specific gene inactivation been foreseen, I am certain it would have been expressly excluded from the Guidelines because altered organisms of this type pose no environmental hazard different in principle from that posed by any ordinary new strain of plant, animal, or microorganism derived through the occurrence of conventional mutations; in fact, the modern variety are much safer because the genetic change is permanent and irreversible, in contrast to classical mutations which can often revert to the wild-type state; indeed, there are cases in which conventional vaccine strains of viruses have reverted to virulence with fatal consequences. This unfortunate possibility is precluded by the modern method of gene inactivation.

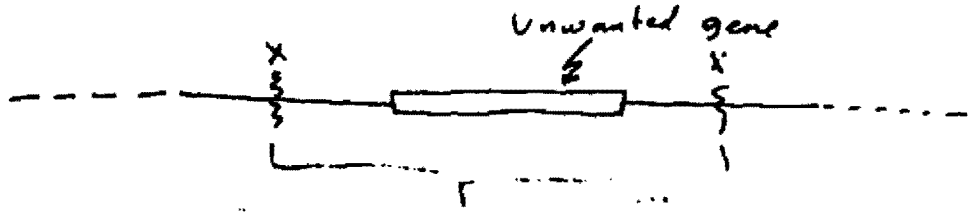
Jeremy Rifkin and the Foundation on Economic Trends appear to act on the basis of a general mistrust of the gene splicing technology and its applications to enforce the letter of the law in a scientifically misinformed manner. The result is inhibition of an entirely non-hazardous and exemplary application of modern biotechnology to real and tractable problems. This type of legalistic

opportunism can be prevented only by rationalizing the regulatory system that has permitted it.

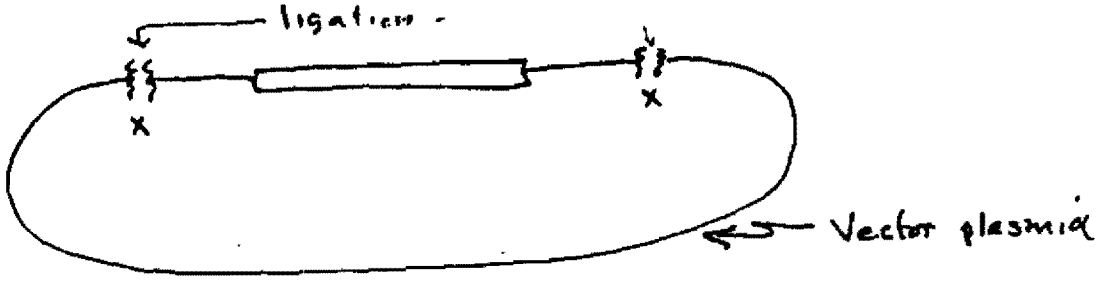
The Recombinant DNA guidelines thus require amendment to exclude experiments in which no foreign genetic material is added to an organism's genetic complement; ideally, such an amendment should precede and then be reflected in any legislative initiatives such as current efforts by Congressman Fuqua and Senator Gore to create a national committee to oversee recombinant DNA policy. Recognizing the genetic distinction between the addition of foreign genes and the removal of native ones would focus on substantive questions of biology rather than on technical details and would unfetter the ingenuity that the new technology allows; hopefully attention could then be directed toward issues of real concern such as the use of gene splicing for biological warfare.

Construction of Specific Gene Deletion

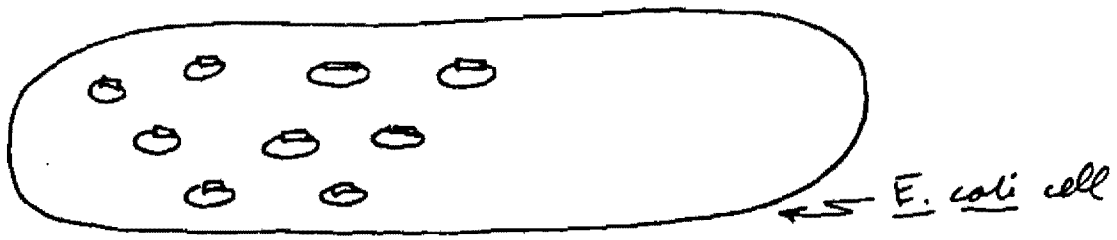
Chromosomal DNA of original organism



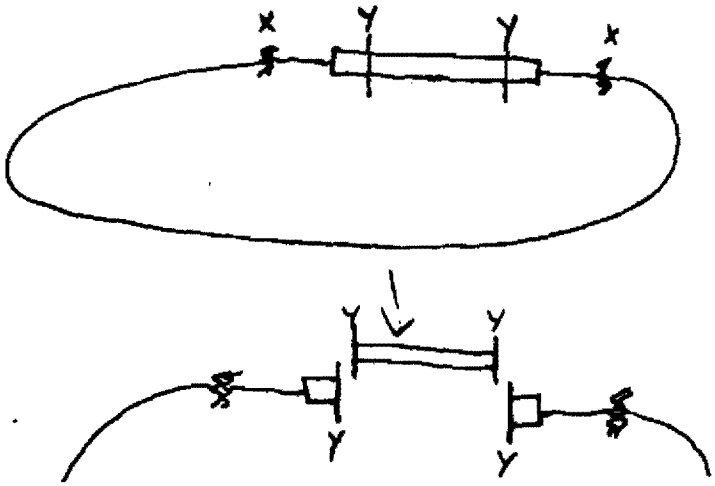
Excised segment, prepared by treating DNA with a restrictive enzyme which makes cuts at specific locations (X)



The excised gene with some extra DNA at each end is then ligated to DNA of a vector plasmid that has been treated with the same restriction enzyme.



The hybrid vector plasmid is transferred to E. coli bacteria to allow multiplication of cloned gene.



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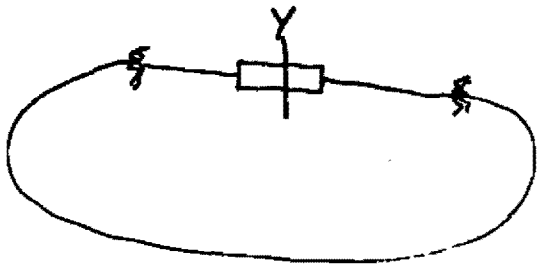
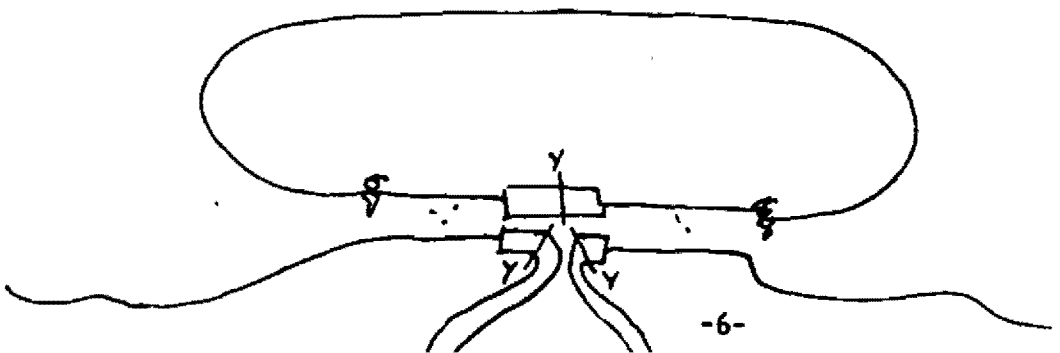
Hybrid plasmid DNA purified, treated with a second restriction enzyme that acts at two sites (Y) within the unwanted gene.



Ligation then ties together the two new ends leaving out the unwanted segment.

This reconstructed hybrid plasmid is then re-transferred to E. coli for amplification, then re-introduced into parent organism, in which it cannot multiply on its own.



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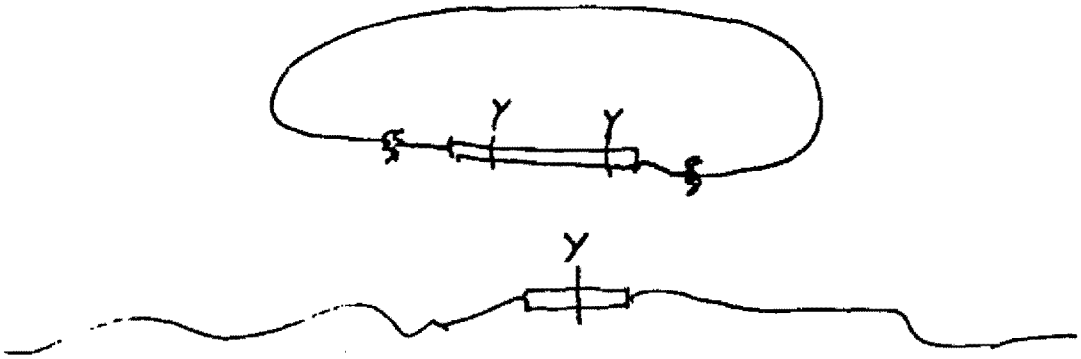
Homologous DNA regions line up; recombination between plasmid and chromosome occurs at x's replacing intact gene with deletion-containing derivative; plasmid, which now contains intact gene again, cannot survive in this organism and is lost.



Net effect is the precise removal of a segment of the native gene, leaving behind no foreign DNA and resulting in no other change in the organism's genetic material.

Note that the biology and biochemistry is such that each step in the outline has a very low probability of occurrence. Splicing technology is totally dependent on the power of microbial genetics, which enables one to select for these rare events.

JAN 6 1987

UNITED STATES DEPARTMENT OF COMMERCE
International Trade Administration
Washington, D.C. 20230

Dr. William J. Gartland
Director, ORDA
Bldg. 31, Room 3B10
National Institute of Health
Bethesda, MD 20892

Dear Dr. Gartland:

After reviewing the proposed changes in the NIH Guidelines for Research Involving Recombinant DNA Molecules, I find the suggested changes to be reasonable.

However, I would strongly urge that in the proposed revision of section III-A-2 of the NIH Guidelines you consider the first alternative, namely to redefine recombinant DNA, rather than to modify section III-A-2.

By redefining recombinant DNA, the guidelines demonstrate support of the definition adopted by the OSTP and strengthen the objective of the OSTP document by demonstrating a coordinated approach.

In contrast, if you become involved with the question of deliberate release, you are opening up a pandoras box for which there are numerous definitions and very little agreement.

Additionally what may be "accepted scientific practice" today may not be tomorrow. I believe that suggestion #2 has the potential of becoming a rather argumentative modificiation

Hope you had a happy new year.

Best Regards,

A. Hellman
Science Advisor
for Biotechnology

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1289 F

COMMENTS ON ITEMS III AND IV PUBLISHED IN FEDERAL REGISTER, PART III,

DECEMBER 19, 1986

ENVIRONMENTAL DEFENSE FUND

257 Park Avenue South
New York, NY 10010
(212) 505-2100

January 21, 1987

Director
Office of Recombinant DNA Activities
Building 31, Room 3B10
National Institutes of Health
Bethesda, MD 20892

Dear Members of the RAC:

Please consider the following comments concerning the proposed changes to the NIH Guidelines for Research Involving Recombinant DNA Molecules (Federal Register 51:45650-3). As section I is a reasonable procedural change, and section II is difficult to assess before Appendices M, N, and O are written, these comments focus on sections III and IV of the proposed revisions.

SECTION III: Working Group Proposition

The Working Group on Definitions presents a proposition--recombinant DNA experiments that do not involve the introduction of foreign DNA should not continue to be subject to regulation as "recombinant DNA"--and two options for implementing it. However, RAC should consider the merits of this proposition before considering its implementation.

The rationale for this proposition is based on laboratory observations of the labile nature of prokaryote genomes. Because DNA deletions and rearrangements are common in laboratory populations, it is assumed that such changes regularly occur in all species in nature. Therefore, the argument goes, releases of comparably altered organisms should not be subject to special scrutiny.

It is necessary to ask, however, whether these laboratory observations accurately portray the genetics of natural populations of prokaryotes. Although laboratory observations led to the notion a few years ago that there might be complete gene exchange among many types of bacteria (see discussion in Selander, 1985), recent studies of the population genetics of bacteria reveal that many populations have high levels of linkage disequilibrium; these populations are collections of independent clones (e.g. Caugent et al.,

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1986; Musser et al., 1985; Selander, et al. 1986; Selander, 1985). Thus the assertion that genetic changes that occur in the laboratory are necessarily commonplace in nature does not hold (Stotzky and Babich, 1984). We do not know rates of genetic flux--especially genetic rearrangements--in nature (let alone how natural selection operates on these changes).

Of course, even if we do not know rates of change, there is no doubt that changes within genomes occur in nature. But, accepting the notion that such genomic changes regularly occur and using this idea to justify releases of engineered organisms are not the same. Two such justifications are standard. The first is the assertion that all prospective genetically engineered organisms exempted by the Working Group's proposition have already occurred, and therefore been "tested," in nature. This cannot be true. For example, it has been estimated that there are 10^{70} atoms in the universe (Ayala and Valentine, 1979). Yet, one organism, heterozygous at only 232 structural gene loci, can produce 10^{70} kinds of gametes. Furthermore, the genotype of a released engineered organism does not fully predict the role the organism will play in the environment it is released into. As experience with introduced species attests, whether or not an organism is historically novel, it can produce novel consequences in a novel environment. Can we believe that any engineered organism covered by this proposition will already have been "tested" in all environments into which it might be released? One might then ask why new evolutionary adaptations ever occur! In addition, natural genetic changes occur in isolated individuals, but releases of engineered organisms will typically involve tremendous numbers of individuals. The ecological effects and viability of such large numbers of individuals may be entirely different than that of an isolated individual. Afterall, epidemiologists know that the spread of microbes depends on the size of source-pools, ecologists understand that many organisms are colonial because of the advantages of living in groups, and evolutionary biologists have established the existance of frequency dependent selection.

The second justification for releases is to assert that since changes within a genome occur naturally, and extensive problems have not resulted from classical breeding programs (although there have been some (Colwell et al., 1985), the releases covered by the Working Group's proposition need no special scrutiny. However, just because an event can potentially occur in nature, does not mean that it should be freely promoted by humans; "natural" is not a justification just as "artificial" should not be a condemnation. For example, invasions of species into novel environments regularly occur in nature; otherwise volcanic islands would not have native faunas and floras, organisms would not currently be recolonizing Mount St. Helens, and much of the Northern hemisphere would not have been recolonized after the last glacial maximum. Yet, USDA wisely does not allow free introduction of organisms into this country.

There is, however, some merit to the above assertion; one can use recombinant DNA techniques to accomplish more precisely genetic changes that could be made through classical techniques. And, <u>on average</u>, the risks of releasing organisms covered by this proposition are likely to be lower than the risks of releasing organisms altered with "foreign" DNA. Nevertheless, it

is premature to exempt all releases involving all classes of genetic change within virtually any genome from all review. (In particular, this exemption includes eukaryotic genomes even though the rationale for it is largely based on prokaryotes.) A low level of review, similar to that outlined in Appendix L, coupled with more specific exemptions that can emerge from experience with, rather than conjecture about, releases is far more appropriate for the organisms covered by the Working Group's proposition. An additional benefit of low level review is that releases would be registered and thus a safety record would develop.

Definition of Recombinant DNA

The definition of recombinant DNA should not be revised so that it includes only organisms altered with "foreign" DNA. The rationale presented at the Working Group meeting, for implementing that group's proposition as a change in definition instead of an exemption, is that recombinant DNA created with "foreign" DNA is different from recombinant DNA created from material within a genome. But of course DNA does not differ among species or strains, and, although exchange within genomes is undoubtedly more common than exchange between genomes in nature, exchange between genomes does occur. Thus the difference between recombinant DNA created with "foreign" and "non-foreign" DNA is quantitative rather than qualitative.

Such a change in definition will have far-reaching and probably unintended effects. Will parts of the Guidelines no longer function as intended? For example, will recombinant pathogens containing no "foreign" DNA be exempt from review? Can any organism with altered genes for toxins or drug resistance be released? (If not, does this mean that the definition of recombinant DNA depends on what organism is genetically altered?) How many rearrangements, amplifications, deletions, and single-base changes can be made and the "same" genome maintained? This last question may seem silly, given the atmosphere of good faith in which RAC operates. But, in order to maintain consistency under the Coordinated Framework for Biotechnology this revised definition would probably also be used for regulatory purposes, in which good faith cannot always be assumed.

In closing, I wish to note that the Federal Register note concerning the Working Group's sentiments about changing the definition of recombinant DNA was misleading. Calling the Working Group "split" as to whether they wished to change the definition of recombinant DNA does not adequately portray the fact that the group voted against changing the definition, 2-7-1.

SECTION IV

Consideration of Section IV raises two questions. First, why should BL1 containment be relaxed for laboratory experiments covered by Appendices C-II, C-III, and C-IV? The BL1 containment guidelines are hardly unreasonable; beyond standard microbiological practices essentially all they stipulate is that laboratories be designed for ready cleaning, pest control be practiced, and any uncontaminated wastes be transported from laboratories in closed containers. Second, why not simply revise these appendices so that unwieldy,

expensive, and unnecessary requirements for large-scale containment are specifically replaced by less stringent requirements, instead of exempting organisms covered by Appendices C-II, C-III, and C-IV from all containment guidelines?

Because these questions are not addressed, the changes proposed in Section IV appear intended to incorporate into the NIH Guidelines the passage from the Coordinated Framework for Biotechnology that states that, "...large-scale containment of many low risk DNA derived industrial microorganisms need be no greater than those appropriate for the unmodified parent organisms," as much as to relieve the fermentation industry of containment responsibilities.

A subsequent passage from the same page of the Coordinated Framework (Federal Register 51:23304) notes that, "By the time a genetically engineered product is ready for commercialization, it will have undergone substantial review and testing during the research phase, and thus, information regarding its safety should be available." Given this point, it seems more appropriate to specify relaxed BL1-LS containment on a case-by-case basis than to completely exempt all organisms covered by Appendices C-II, C-III, and C-IV--including untested organisms in the research phase--from the NIH Guidelines. A list of engineered organisms currently employed by the fermentation industry to which relaxed containment guidelines apply could be added to Appendix C.

Thank you for your attention.

Yours truly,

Rebecca J. Goldburg

Rebecca J. Goldburg, Ph.D.
Staff Scientist

References

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ABBOTT

Corporate Quality Assurance

Abbott Laboratories
Abbott Park
North Chicago, Illinois 60064, U.S.A.

January 21, 1987

Director, Office of Recombinant DNA Activities
Building 31, Room 3B10
National Institutes of Health
9000 Rockville Pike
Bethesda, MD 20892

Dear Sirs:

We wish to comment upon proposals III and IV, No. 244, p.45651, as proposed actions under the NIH Guidelines for research involving recombinant DNA molecules.

III. Proposed Revision of Section I-B or Section III-A-2 of the NIH Guidelines

We strongly support the proposal to modify the NIH Guidelines to exempt DNA experiments which do not involve the introduction of foreign DNA. Option 1 is preferable in that it will clarify the concept of exempting experiments not involving foreign DNA, by stating a definition of what constitutes recombinant DNA, and will refocus the NIH Guidelines to those areas of research which may, by their nature, require oversight of the Institutional Biosafety Committee and the RAC of the NIH.

IV. Proposed Revisions of Appendices C-II, C-III, and C-IV

As a major member of the fermentation industry, we applaud the proposed action to treat the large-scale fermentation containment under appendices C-II, III and IV the same as the fermentation containment for the host organism. This is appropriate given the experience of the fermentation industry and the experience gained working with these recombinant organisms.

We appreciate the opportunity to comment upon these proposals and urge the RAC to act positively upon them.

Sincerely,

C. Searle Wadley

C. Searle Wadley, Chairman
RDNA Biosafety Committee

John H. Keene

John H. Keene, Dr.P.H.
Secretary and Biological Safety Officer
RDNA Biosafety Committee

CSW/JHK/pc

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uwbc

University of Wisconsin Biotechnology Center

Dr. Richard R. Burgess
Director, UWBC
1710 University Avenue
Madison, WI 53705

January 19, 1987

Director
Office of Recombinant DNA Activities
Building 31, Room 3B10
National Institute of Health
Bethesda, MD 20892

Dear Sir,

I am writing in support of the Proposed Amendments of NIH Guidelines (51 FR 45650-45652). RAC has, in my opinion, served the citizens, scientists and businessmen of this country well by taking a cautious position with regard to the safety of recombinant DNA research and then relaxing the guidelines when experience shows that to be warranted. The proposed amendments, especially III and IV, are in that tradition. Amendment IV will have a tremendous positive effect on research and development at the University of Wisconsin, especially on projects associated with the Biotechnology Center. In addition, the biotechnology industry will be able to manufacture recombinant DNA derived protein products more efficiently and cheaply.

I hope this evaluation and review by RAC will continue and that other regulatory agencies will also be cautious but reasonable.

Sincerely yours,

Richard R. Burgess

RRB:jas

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1289 G

COMMENTS ON ITEM IV PUBLISHED IN FEDERAL REGISTER, PART III, DECEMBER 19, 1986

HOFFMANN-LA ROCHE INC.

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Drug Regulatory Affairs
(201) 235-5000

January 20, 1987

Director, Office of Recombinant
DNA Activities
National Institutes of Health
Building 31, Room 3B10
8800 Rockville Pike
Bethesda, Maryland 20892

Gentlemen:

Hoffmann-La Roche Inc. would like to provide the following comments and recommendations to the notice published in the Federal Register, Vol. 51, No. 244, Friday, December 19, 1986.

Proposed Revisions of Appendices C-II, C-III, and C-IV

We propose the following rewording of the two paragraphs on page 45652 of the Federal Register notice; the new paragraph should read:

> "The appropriate physical containment conditions need be no greater than those of the host organism unmodified by recombinant DNA techniques for fermentation and the subsequent processing of fermentation broths and cell pastes at laboratory or production scale for host vector systems that are exempt from these Guidelines."

The above modifications further clarify the word "experiments" and allow the processing of live cells in an uncontained mode. It is especially important to include research experiments and production activities, since other sections of the NIH Guidelines and amendments to the Guidelines reference "manufacture" of DNA-derived pharmaceuticals approved by the Food and Drug Administration.

Sincerely,

HOFFMANN-LA ROCHE INC.

Linda S. Dujack

Linda S. Dujack, Ph.D.
Associate Director
Drug Regulatory Affairs
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LSD:gm
HLR No. 87053

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Copy to: Dr. William Szkrybalo (PMA)

THE UPJOHN COMPANY

KALAMAZOO, MICHIGAN 49001 U.S.A.
TELEPHONE (616) 323-4000

Theodore Cooper, M.D., Ph.D.
Vice Chairman
(616) 323-7095

January 19, 1986

Director
Office of Recombinant DNA Activities
Building 31, Room 3B10
National Institutes of Health
Bethesda MD 20892

Dear Sir:

The Upjohn Company strongly supports the proposed revisions in Appendices CC-II, C-III and C-IV of the National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules (*Federal Register*, Vol. 51, No. 24, December 19, 1986, pp.45650-45652). The revisions proposed by the Commissioner of the Food and Drug Administration are progressive and significantly clarify the appropriate containment for large-scale recombinant fermentation experiments. These revisions will make it easier for industry to engage in strategic planning. They also bring into focus the safe history of the fermentation industry and the generally innocuous natures of microorganisms used to produce antibiotics, proteins, amino acids and vitamins. All evidence accumulated to date on *Escherichia coli K-12*, *Bacillus subtilis* and *Saccharamyces cerevisiae* support their inclusion in the classification of non-pathogenic and innocuous microgranisms. The introduction of foreign genetic information into such organisms does not change their natures unless the foreign DNA encodes for biosynthesis of toxic molecules or antibiotic resistance, as described in Sections III-A-1 and III-A-3 of the Guidelines.

Finally, the Commissioner addresses the important economic aspects of developing and applying recombinant DNA technology. If the United States is to achieve significant commercialization of this technology, the capital costs of large-scale recombinant processes must be competitive with both foreign-based recombinant process and conventional domestic ones. The proposed changes will help in this regard, and they will allow the United States to retain its role as the world's leader in biotechnology.

The proposed changes in Appendices C-II, C-III and C-IV are progressive in both a scientific and an economic sense, and they will not put the public at any greater risk. We recommend their adoption.

Sincerely,

Theodore Cooper

Theodore Cooper, M. D., Ph.D.
Vice Chairman of Board of Directors

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GRACE

Vincent F. Simmon, Vice President
Research Division

W.R. Grace & Co.
7379 Route 32
Columbia, Maryland 21044

(301) 531-4417

January 19, 1987

Dr. Bernard Talbot
Deputy Director
National Institute of Health
9000 Rockville Pike
Bethesda, MD 20892

Dear Dr. Talbot:

We are in favor of the clarification language proposed as an amendment to the NIH Guidelines in Appendices C-II, C-III, and C-IV.

Respectfully yours,

Vincent F. Simmon, Ph.D.
Vice President

VFS:csc

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UNIVERSAL Foods CORPORATION

GARY W. SANDERSON, PH.D.
VICE PRESIDENT-RESEARCH

January 15, 1987

The Director
Office of Recombinant DNA Activities
Building 31, Room 3B10
National Institutes of Health
Bethseda, MD 20892

Dear Sir:

I am writing to comment on the Notice "Recombinant DNA Research: Proposal Actions Under Guidelines" published in the Federal Register on December 19, 1986 (51 FR 45650-45652).

I heartily support all of the proposals listed in this Notice, but I particularly want to support the changes in the "NIH Guidelines for Work with Recombinant DNA Organisms" that are proposed by Dr. Frank E. Young, Commissioner of Food and Drugs (51 FR 45651, Column 3, to 51 FR 45652, Column 2). Dr. Young's comments state the justification, and the need, for relaxing the NIH Guidelines to allow safe recombinant organisms to be cultured in the same way that one cultures organisms of the same type that are genetically unmodified by recombinant DNA techniques. Dr. Young's comments are concise and they are accurate; and they deserve to be supported.

My company (Universal Foods Corporation) is one of the world's largest manufacturers of baker's yeast (Saccharomyces cerevisiae) and other yeast products. Our brand name for yeast products is "Red Star™," and we manufacture and market these products in nine countries around the world in addition to the United States. This product has been consumed as a food, and as a constituent of food products, by people the world over for centuries. Without exception, baker's yeast in all of its forms is recognized as a wholesome food material that imparts aesthetically pleasing qualities to many food products (especially flavor and leavening to bakery products). And, various forms of the yeast Saccharomyces cerevisiae (such as baker's yeast and brewer's yeast) are also recognized to be important sources of vitamins and minerals, and they are consumed for these beneficial constituents by many people.

There is certainly unanimous agreement that the yeast Saccharomyces cerevisiae is a safe organism. And the insertion of genetic material for safe proteins, and for enzymes that promote innocuous reactions, into the yeast Saccharomyces cerevisiae does not change the safe nature of the original organism. For instance, in our laboratory, we have inserted genes for "lactose permease" and for "beta-galactosidase" from the yeast Kluyveromyces lactis into a baker's yeast strain of Saccharomyces cerevisiae in order to

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TECHNICAL CENTER
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prepare a baker's yeast that can be grown on lactose which is available in cheese whey, a byproduct of cheese manufacturing. There is nothing unsafe about either yeast strain involved in this product of recombinant DNA work, and the new properties of the recombinant DNA baker's yeast strain are entirely innocous. There is no conceivable reason why this new, genetically engineered baker's yeast should not be considered to be as safe as any baker's yeast that is unmodified by recombinant DNA techniques.

We are also working with a strain of the yeast Saccharomyces cerevisiae that has been modified by recombinant DNA techniques to produce the human protein "alpha-1-antitrypsin." In this case, it is inconceivable that a health hazard exists from contact with this yeast, and it is virtually impossible for this yeast to compete in open environments because of the useless metabolic load imposed by the "alpha-1-antitrypsin" gene. Again, there is no reason not to handle this recombinant yeast strain in the same way that one handles strains of the yeast Saccharomyces cerevisiae that are not modified by recombinant DNA techniques.

Above are two examples of the yeast Saccharomyces cerevisiae modified by recombinant DNA techniques that are certainly as safe as strains of the yeast Saccharomyces cerevisiae that are not modified by recombinant DNA techniques. It should certainly be concluded that these new genetically engineered strains of yeast should be allowed to be handled by the same methods used for strains of Saccharomyces cerevisiae that are not genetically modified by recombinant DNA techniques. And this conclusion should be generalized as Dr. Frank E. Young, Commissioner of Food and Drugs, has recommended.

In conclusion, I would like to point out that there has not been even one unpredicted product of genetic modification of organisms by recombinant DNA techniques in the entire world. This record stands even after more than a decade of thousands of experiments in hundreds of laboratories around the world. Certainly, thousands of people have been exposed to such genetically modified organisms for hundreds of hours as a result of all this work, and surprising adverse results have never been recorded. It is time to acknowledge that modification of organisms by recombinant DNA techniques produces organisms that are no more dangerous, nor more safe, than the organisms from which the genetically modified organism was derived. And this fact should be reflected in the Guidelines, and the Regulations, that pertain to any aspect of recombinant DNA work with organisms.

I do hope that the recommendations proposed in the referenced Notice are accepted and that the NIH Recombinant Advisory Committee Guidelines are amended accordingly.

Sincerely yours,

Gary W. Sanderson, Ph.D.
Vice President, Research

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GWS/slb

1289 H

COMMENTS ON ITEMS I, II, AND III PUBLISHED IN FEDERAL REGISTER, PART III,

DECEMBER 19, 1986

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OF COUNSEL:
SANDRA E. HAMILTON
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January 28, 1987

Director
Office of Recombinant DNA Activities
Building 31, Room 3B10
National Institutes of Health
Bethesda, Maryland 20892

Re: Comments on proposed Revisions to Guidelines, 51 Fed. Reg. 45650 (December 19, 1960)

These comments are submitted on behalf of the Foundation on Economic Trends and Jeremy Rifkin. Paragraph numbers herein coincide with those of the Notice.

I. The difficulty with the proposed change is that, while it is intended to cover the situation where both NIH and another agency have jurisdiction to review the experiment, it fails to suggest any mechanism for resolving the overlap in a prudential, discretionary manner. Instead, the proposal simply calls for NIH to abdicate its role, regardless of whether NIH is satisfied that the review by the other agency "serves the same purpose" as that which would be conducted by the RAC and NIH, is of comparable quality, or that the other agency, rather than NIH, has the most appropriate expertise to bring to bear on the particular questions raised by the experiment.

Instead of the withdrawal of NIH review-decision authority as proposed, we suggest that after preliminary review by ORDA and by the other agency (or agencies) of the nature of the proposed experiment, that they confer and decide -- on the basis of appropriate criteria such as expertise and capability -- which agency is to conduct the review. It may be that through a memorandum of agreement with other agencies, those decisions can be made in advance for certain categories of experiments. It most instances, even those where the other agency must issue a permit, license, or approval, it will neverthless be desirable that the RAC-NIH review-approval process be implemented, and that the other agency have the benefit of that review and decision.

A vivid example of the need for NIH review and oversight is the situation involving the conduct of field tests for a

pseudorabies vaccine at Baylor College of Medicine and/or Texas A & M University (tests conducted by Dr. Saul Kitt and Novagene, Inc., with participation by USDA) in violation of the NIH Guidelines. See Memorandum dated October 9, 1986, from Director, NIGMS, to Director, NIH. It is certainly preferable to have the RAC-NIH review conducted before, rather than after, licensing, approval, and marketing of a product by another agency, as occurred in that instance.

While it would be desirable that other federal agencies have the experience and expertise that NIH has in reviewing recombinant experiments, the fact is that many of them do not. Yet at this time, there is a growing need for such expertise. The proposal in question, then, is counter-productive at this time, for assuring adequate and timely review of proposed experiments. Nothing should be done at this time to encourage researchers to bypass the IBCs or RAC where they may well prove to be the most appropriate reviewing institutions.

II. Because of the overlapping nature of the proposals in part II and III of the Notice, certain of the comments herein also refer to segments of part III.

As a preliminary matter, each of the proposed revisions to Section III-A-2 of the Guidelines attempt to define "deliberate release" as "a planned introduction . . . into the environment." The difficult questions -- alluded to, but not resolved in the publication entitled "Coordinated Framework for Regulation of Biotechnology," 51 Fed. Reg. 23302 (June 26, 1986) -- as to when and under what circumstances an organism (however defined) may be deemed to have been introduced "into the environment" are not addressed in the Notice in question. The several federal agencies dealing with this issue may well have differing views as to its resolution. For example, EPA's definition of deliberate release for purposes of the superfund law (CERCLA) may be different from that deemed appropriate by NIH or USDA.

The definition of "deliberate release" as determined by the meaning of the phrase "into the environment" will be of great significance in many instances in determining whether a deliberate release is involved. Inasmuch as NIH is attempting to coordinate its review/decision efforts with those of other agencies (as is reflected, for example, in proposal I in the Notice discussed above), and that both NIH and applicants need guidance as to the applicability of Section III-A-2, that definition is of practical importance.

We therefore suggest that NIH address that question as part of its proposed amendments by developing, at this time, general

criteria as to the meaning of "into the environment" in coordination with other federal agencies. While, as is true of many regulatory situations, that criteria may have to be modified as experience dictates, it can be made sufficiently flexible to include all experiments that must be reviewed and, at the same time, provide much needed guidance.

Turning to the RAC Working Group recommendation developed on December 5, 1986, a fundamental problem with proposed Section III-A-2 and subsection a thereof (in both parts II and III of the Notice) is that the essence of the change from the current III-A-2 is the expansion of the exemptions as will be established by the evidence to be described in Appendices L, M, N and O. With the exception of existing Appendix L for certain plants, the other appendices are not yet developed. Therefore, at this time, there is no basis whatsoever for approving their creation or for making the other proposed changes in III-A-2 and developing subsection a to accomodate them. In short, the proposal is premature.

We request that if and when it is decided to develop the appendices, there be full and adequate represention of the wide variety of disciplines relevant to that undertaking among the voting members of the working group or committee assigned that responsibility, including micro-ecologists and other ecologists.

Our other comments relating to part II are discussed below.

III. We oppose both options submitted by the Working Group. While the Notice states that "[t]he working group were split as to whether they preferred dealing with this problem by changing the definition of recombinant DNA or by further modification of other sections of the Guidelines," the overwhelming majority voted against changing the definition (Option 1) by 7 to 2 with 1 abstention.

One obvious problem with Option 1 is that it would mean no NIH review of deletions and rearrangements within the human genome.

A problem with both options (and with proposed Section III-A-2 and subsections a and b under II), is that the mutations included within subsections b and c can present serious risks of adverse ecological and health effects. Some of these problems were described by Dr. Frances Sharples, a member of the Working Group, at the most recent meeting of the RAC.

The significant and continuing controversy over these (and similar) proposed taxonomic definitions as a basis for

determining the extent and nature of regulatory review is well documented, both within the federal agencies and by the comments and concerns of outside experts. See, e.g, "Summary: EPA Biotechnology Workgroup Retreat," July 31, 1985, pp. 2-3; "Briefing Materials" for "Briefing for Jack Moore OTS Biotechnology Issues," August 5, 1985 (section dealing with "Issue: What Commerical Products Should We Review, i.e., What is 'New' Under TSCA"); "OPTS Biotechnology Issues for Assistant Administrator Resolution," July 1985 (Draft, July 24, 1985) (EPA), pp. 7-9; "Review of Draft Federal Register Notice on Biotechnology" (Work Assignment No.: L-86-10/28-09) Work Assignment Title: Expert Review of Biotechnology Proposal, Work Assignment Reports (Dec. 6, 1985) (EPA) -- Work Assignment Report by Dr. Dorothy Jones ("It is not true that genera are stable. . . . If the terms intra- and inter-generic, which occur throughout the policy statement, are removed (and in my opinion that should be) one is left with the repetition of the rather clumsy 'similar' and 'dissimilar' organisms, but to my mind this is a better solution. It would be quite wrong to include in the document a statement which is just not true." (Pp. 2-3; see also pp. 4-8)); Work Assignment Report by Dr. Bruce R. Levin ("I believe that the inter-'genus' criteria for regulated genetic manipulation is, in the cases of microbes, somewhat arbitrary. . . . I also believe that there are problems with the pathogen, non-pathogen criteria for regulation" (p. 3) (and see his more expansive comments on the same points at pp. 5-7); Work Assignment Report by Dr. Daniel Simberloff ("It is odd to view deletion products as not having new combinations of genetic material. . . . [A] deletion could quite readily combine traits that are not normally found together." (p. 3) (and see his comments about the "degree of circularity in this [inter-intra-generic] distinction, which is the linchpin of the entire proposal" and that "the way around this is to emphasize phenotypes more." (p. 4)); Work Assignment Report of Dr. Max Summers (". . . it is difficult to predict how sound EPA's proposed policy concerning the relative hazards of inter-or intragenic combinations are since there is very limited experimental data availabe to assess this judgement in an environmental context. . . . I think there will be many exceptions to the rule." (p. 1); "I would be reluctant to advise on the validity of EPA's proposed policy. It makes more sense to base the evaluation upon the nature of the gene/function/trait which is of question." (p. 2)); Memorandum (EPA) dated March 26, 1985, "Subject: Comments on BSCC Definition of 'Inter-Generic Microorganism,' 'Pathogen' and 'Environmental Release'" from Don Clay, Director, Office of Toxic Substances to John A. Moore, Assistant Administrator for Pesticides and Toxic Substances; [Prepared] Testimony of Elliott A. Norse, Ph.D., Director, Public Affairs Office, The Ecological Society of America on The

Coordinated Framework for the Regulation of Biotechnology before the U.S. House of Representatives Committee on Science and Technology Subcommittees on Investigation and Oversight, Natural Resources, Agriculture Research and Environment and Science, Research and Technology (July 23, 1986).

Because of the concerns expressed by these and other persons highly knowledgable in the field that the exemptions proposed in the Notice are not scientifically well founded, and may lead to the inadvertent failure to review and regulate experiments with serious risks, we request that those exemptions not be adopted.

Sincerely yours,

Edward Lee Rogers
Counsel for Foundation on
Economic Trends and
Jeremy Rifkin

PROPOSED AMENDMENT TO TALBOT PROPOSAL (TAB 1283)

Add the following sentence after the second sentence, which concludes with the words "...may proceed without the necessity for NIH review or approval":

> "However, any experiment that involves the administration of gene therapy to human subjects (see Section III-A-4 of the Guidelines) may not proceed without prior review by the NIH Recombinant DNA Advisory Committee and NIH approval."

Submitted by LeRoy Walters

February 2, 1987

United States Department of Agriculture

Animal and Plant Health Inspection Service

Washington, D.C. 20250

January 29, 1987

Dr. William Gartland, Director
Office of Recombinant DNA Activities
Building 31, Room 3B10
National Institutes of Health
Bethesda, Maryland 20892

Dear Dr. Gartland:

We wish to take this opportunity to respond to "Recombinant DNA Research: Proposed Actions Under NIH Guidelines" which appeared in the Federal Register on December 19, 1986, (51 FR 45650-52).

We support the proposed deletion of the current language in Section III-A and favor the proposed addition of the described paragraph at the end of Section I-A of the Guidelines. While the proposed change would continue the National Institutes of Health (NIH) review requirement for experiments covered by Section III-A of the Guidelines, it would eliminate NIH review for all other DNA experiments because the review would be conducted by another Federal agency. Such a change would be consistent with the development of regulatory authority in Federal agencies in response to the expanding commercialization of products derived from biotechnological procedures. This proposed change would also help eliminate possible confusion about the agency to which an experiment should be submitted for review and approval.

In Section III-A of the current Guidelines, experiments which require specific NIH-Recombinant DNA Advisory Committee (NIH-RAC) review and approval by both NIH and the Institutional Biosafety Committee (IBC) may be submitted to another Federal agency. If the NIH Office of Recombinant DNA Activities (ORDA) determines that such reviews serve the same purpose, NIH approval is unnecessary and the proposed experiment may be initiated with approval from the other Federal agency. At the present time, there are no provisions or requirements for the transfer of such information between the NIH and another Federal regulatory agency. Because the review by another Federal agency serves the same purpose as that currently conducted by NIH, it would be redundant to require overlapping reviews. Each regulatory Federal agency may also require unique criteria not normally required by NIH.

Currently, the NIH Guidelines provide the conditions under which only plants containing recombinant DNA molecules may be released in the environment. The RAC Working Group on Definitions, at their meeting on December 5, 1986, recommended the establishment of new appendices, similar to Appendix L "Release Into the Environment of Certain Plants" which would be written to include conditions of release for animals, microorganisms other than vaccines

and vaccines. We favor the proposal by the RAC's Working Group on Definitions to amend Section III-A-2 by adding parallel sections to be written as Appendices M, N. and O covering respectively animals, microorganisms, other than vaccines, and vaccines. We also urge appropriate Federal, private, and public involvement in the preparation of the criteria for these new Appendices.

During the December meeting, the RAC Working Group on Definitions passed a motion concerning changing the definition of recombinant DNA. It was felt that certain types of such experiments which do not include the introduction of foreign DNA need not be subjected to these Guidelines. Of the two options presented, we favor option 2 for the following reasons. The proposed modification of Section III-A-2 provides clear, concise and much needed clarification of the concept that deliberate release is essentially a dangerous event. The proposed use of describing such releases as "planned introductions" under accepted scientific practices in which there is adequate evidence of biological and/or physical control of the recombinant organisms is consistent with Departmental, environmental, and safety concerns. Although the proposed changes would exempt experiments involving deletion derivatives, single base changes, rearrangements and amplifications within a single genome, these same types of experiments would still require other Federal agency review and approval before release from containment.

The final proposal in this notice deals with reducing the physical containment requirements for low risk microorganisms used in industrial fermentations. We support Dr. Frank Young's proposal to reduce unnecessary containment procedures currently described in BL1-LS for such large scale fermentations. We feel that the containment conditions need to be no greater than those employed for unmodified host organism experiments.

Sincerely,

Bert W. Hawkins
Administrator

Dist.

PUBLIC AND SCIENTIFIC AFFAIRS BOARD

AMERICAN SOCIETY FOR MICROBIOLOGY

1913 I STREET, N.W.
WASHINGTON, D.C. 20006
TELEPHONE: (202) 822-9229

January 29, 1987

Dr. William Gartland
Director, Office of Recombinant DNA Activities
Bldg. 31, Room 3B10
National Institutes of Health
Bethesda, MD 20892

Dear Dr. Gartland:

On behalf of the American Society for Microbiology (ASM), we are submitting the following comments in response to the proposed actions involving the National Institutes of Health (NIH) Guidelines for Recombinant DNA Research, published in the Federal Register of December 19, 1986 (51:244). The ASM is the largest single biological life science organization in the world with an active membership of over 34,000. The ASM membership includes scientists from the government, academe and industry, who are experienced in molecular biology and genetics, environmental microbiology, microbial physiology, agricultural and industrial microbiology.

I. The ASM supports adoption of the revisions proposed by Dr. Bernard Talbot to amend Sections I-A and III-A of the NIH Guidelines. This proposed revision is consistent with the policies established by the June 26, 1986 "Coordinated Framework for Regulation of Biotechnology," and will clarify for submitters that recombinant DNA experiments requiring approval under the NIH Guidelines need not be reviewed by the NIH Recombinant DNA Advisory Committee (RAC) once review and approval has been given by another agency with the appropriate jurisdiction.

II. The ASM supports adoption of the revisions, proposed by the RAC Working Group on Definitions to Section III-A-2 of the NIH Guidelines, which define deliberate release and clarify conditions under which a deliberate release experiment would be exempt from review by RAC. We believe these revisions represent the proper approach to dealing with the issue of deliberate release and will assure proper planning and participation by scientists in the decision-making process.

III. The ASM agrees with the motion passed by the RAC Working Group on Definitions "that certain types of recombinant DNA experiments which do not involve the introduction of foreign DNA need not be subjected to special regulation as 'recombinant DNA.'" We endorse the second option for dealing with this problem by further modifying Section III-A-2 of the NIH Guidelines. Under this option, deliberate release experiments involving genetically engineered organisms created by deletions, single base changes, rearrangements and amplification within a single genome would be exempt from RAC review. We believe the current definition of recombinant DNA should remain unchanged and the RAC should continue its past practice of recommending exemptions.

IV. The ASM concurs with the proposed revisions of Appendices C-II, C-III and C-IV. We believe that containment for low-risk microorganisms should be minimal and endorse the proposed change with the understanding that it does not include organisms otherwise covered under the guidelines and that the experiments performed will be consistent with good laboratory or manufacturing practice.

We appreciate the opportunity to comment on these proposed actions under the Guidelines for Recombinant DNA Research.

Sincerely,

Jean E. Brenchley

Jean E. Brenchley, Ph.D.
President, American Society for Microbiology

Harlyn O. Halvorson

Harlyn O. Halvorson, Ph.D.
Chairman, Public and Scientific Affairs Board

Kenneth I. Berns

Kenneth I. Berns, M.D., Ph.D.
Chairman, Committee on Medical Microbiology and Immunology

Rudy J. Wodzinski

Rudy J. Wodzinski, Ph.D.
Chairman, Committee on Agricultural, Food and Industrial Microbiology

Dist.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

Mr. Robert M. Mitchell
Chair, Recombinant DNA Advisory Committee
National Institutes of Health
9000 Rockville Pike
Bethesda, Maryland 20892

Dear Bob:

As you know, a subcommittee of the Environmental Protection Agency (EPA) Biotechnology Science Advisory Committee (BSAC) was convened on December 11-12, 1986, to attempt to develop several approaches to defining "release to the environment" of microorganisms.

I'm sending you a short draft interim report on that meeting to facilite an exchange of information between the Recombinant DNA Advisory Committee (RAC) and the BSAC. The full report of the committee will be available in early 1987.

I will continue to keep you informed of BSAC activities in this and other areas of mutual interest.

I'd like to take this opportunity to wish you a prosperous and happy 1987.

Sincerely yours,

Elizabeth

Elizabeth Milewski Ph. D.
Executive Secretary
Biotechnology Science
Advisory Committee

ENVIRONMENTAL PROTECTION AGENCY

INTERIM SUMMARY REPORT

THE DECEMBER 11-12, 1986 MEETING OF THE SUBCOMMITTEE ON DEFINITION OF RELEASE TO THE ENVIRONMENT OF THE BIOTECHNOLOGY SCIENCE ADVISORY COMMITTEE

February 1987

Background

The use of the techniques which have been called the "new" biotechnology (recombinant DNA, cell fusion, etc.) has brought to the fore certain problems in assessing the potential impacts of the technology. Among the issues are what constitutes "contained" and "released" to the environment when microorganisms are used to perform certain tasks.

Because the manner in which the Environmental Protection Agency (EPA) will regulate certain products of biotechnology is, in large part, dependent upon whether a product is "released" to the environment, a workable definition of "released" is needed. Such a definition will permit both industry and EPA to determine whether a particular use of a microbial product constitutes a release to the environment subject to a level of regulatory oversight.

In order to tap a broad spectrum of expertise in its efforts to develop a definition of "released" which can be used to regulate certain microbial products of biotechnology, EPA assembled a group of recognized technical experts as a subcommittee of an Agency-based scientific advisory committee. This subcommittee, the Subcommittee on Definition of Release to the Environment of the Biotechnology Science Advisory Committee (BSAC), met on December 11-12, 1986, in Crystal City, Virginia. A subcommittee roster is attached.

Meeting Format

On the first day of the meeting, the issues and the goals of the meeting were explained. Specific comments and observations were solicited from each participant from the perspective of his expertise and research experience. The group was then asked to "brainstorm" and attempt to suggest as many approaches as possible. The subcommittee was asked at this point not to judge the acceptability or credibility of the approach.

After several approaches were suggested, the subcommittee members were assigned to small groups to draft specific language for the proposed approaches. The subcommittee was later asked to comment on the advantages and disadvantages of the approaches.

A complete report of the meeting will be available from EPA in the near future.

Agency Use of the Suggested Approaches

The approaches will be analyzed by the Agency on the basis of several criteria: scientific credibility, legal, policy, and economic implications, resource implications for both the Agency and the industry, ease of implementation, and technical

feasibility. The results of this analysis will be published for public comment as part of the Agency's rulemaking process. After consideration of public comments and regulatory and policy issues, EPA will issue final rules incorporating a definition of "release" to the environment. This definition of release will apply to environmental applications of microbial pesticides under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) and to environmental releases of microorganisms subject to the Toxic Substances Control Act (TSCA).

Suggested Approaches to Defining Environmental Release

Briefly summarized, the approaches to defining release suggested by the subcommittee are:

#1

This approach proposes that a number of organisms released to the environment would trigger EPA review. For regulatory purposes a "release" would be greater than 10^X of a particular organism, where 10^X is the number of organisms released per day from a greenhouse built and operated according to specifications found in a "good greenhouse practices" greenhouse.

#2.

This approach was initiated as an attempt to base a definition of release on "use" or application in industry; e.g., in the mining industry or in agriculture. However, it is difficult to develop a definition specifying use because of the many potential uses, both known and unknown, of microorganisms. As a derivative of this approach, a stage of product development was specified as the trigger for EPA regulation. All product development proceeds through several stages: from laboratory (bench scale), to prototype (pilot scale), to product, to commercial use. The move to pilot scale testing would be the trigger for EPA review. Pilot scale is defined as a limited discharge in a single geographic area. The size of "pilot scale" would vary from industry to industry. In the mining industry, pilot scale would be 1000 tons of rock a day. In the oil recovery business, pilot scale would be 25,000 to 50,000 barrels of oil a day. In the pesticide industry, 10 to 100 liters per day is considered pilot scale.

3.

This approach, based on "control methods", employs a point scheme. Points would be assigned to biological and physical control measures. Examples of control measures include: certain features of the organism; various physical barriers; or remedial activities. Through addition, subtraction,

or multiplication, the points assigned to the control measures would be summed to obtain a point total. EPA would select the point total which would trigger EPA review.

4.

This approach, based on a concept of containment, specifies that EPA notification and review will occur prior to conducting any testing or procedure in an "unrestricted environment", regardless of the size of the test or the procedure.

5.

This approach is based on an evaluation of risk as a trigger for EPA review. Under the risk-based approach, low, medium and high risk ratings would be applied to "factors" in three categories: (1) biological; (2) applications/use; and (3) environmental.

Examples of biological factors include: number of organisms released; genetic stability; pathogenicity; survivability; controllability; host range; and origin (indigenous versus exotic).

Examples of the application/use criteria include: manufacturing; biorational (biocontrol); oil recovery; agricultural (pesticide or fertilizer); and metal reclamation.

Examples of environmental criteria include: laboratory; greenhouse; field; commercial production facility; fresh water systems; and marine water systems.

A "point scheme" might also be applied to this approach.

6.

This approach is an attempt to utilize categories of organisms to define release. Under this approach, review would be required for certain categories of organisms: for example, all pathogens, organisms placed in a new niche, or for new use of an organism.

7.

This approach combines a containment and an organism based approach. A chart would be constructed where containment levels would be on the horizontal axis, while category of organism would be on the vertical axis. Review would be triggered for certain categories of organisms at certain levels of containment.

Five levels of containment were envisaged. The highest level of containment would prevent release of most organisms. The next level of containment would be similar to the highest level of containment except that the performance standards would be lower. The third category would be a "shielded" type of containment; i.e., a setting buffered from the natural environment by means of some type of physical separation from niches where the organism might be able to grow and survive. The traditional greenhouse might fall in this category. The fourth level would be a setting, such as a field plot, where the release can be mitigated by chemical or physical means. The fifth level would be a setting, such as a field plot, limited by some restriction such as an acreage limitation.

Categories of organisms would have to be developed. Examples of organism categories include: animal pathogens, plants pathogens, saprophytes.

ROSTER FOR THE BSAC SUBCOMMITTEE ON ENVIRONMENTAL RELEASE

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REPORT OF THE WORKING GROUP OF THE HUMAN GENE THERAPY SUBCOMMITTEE ON A GENERAL INFORMATION DOCUMENT

RAC Meeting February 2, 1987

The Working Group of the Human Gene Therapy Subcommittee has taken on the job of putting together a brief explanatory document for the benefit of the general public.

The purpose of the document is to afford the non-scientific public an understanding of Human Gene Therapy. The document is largely based on "Points to Consider in the Design and Submission of Human Somatic-cell Gene Therapy Protocols".

On January 9th. the following people met at the HIH:

Dr. LeRoy Walters-- of the Center for Bioethics, Georgetown University, and Chairman of the Human Gene Therapy Subcommittee.

Dr. Maurice Mahoney -- of the Department of Genetics at Yale University.

Dr. Robert Rich -- of the Institute of Government Public Affairs at the University of Illinois.

Attorney Judith Areen -- of the Georgetown University Law Center.

Dr. William Gartland -- Executive Secretary of RAC.

Dr. Henry Miller -- of the Food and Drug Administration.

Dr. Robert Wieder -- of the Heart,Lung and Blood Institute. and I, Anne Witherby -- public representative.

We discussed the scope of the project which was to write what was originally referred to as a "Lay Summary" of the "Points to Consider". The following are a few of our conclusions:

We will make an effort to limit the document to two or three pages which could be attached to the "Points to Consider".

We think the two or three sheets document could also be mailed separately and that it might, at some future time, be enlarged and elaborated into material for a broad variety of educational purposes.

We suggest the title of; OVERSIGHT OF RESEARCH INVOLVING GENE THERAPY FOR HUMAN PATIENTS - GENERAL INFORMATION.

We plan to divide the paper into four sections.

The first, an Introduction, will include an explanation of Human Gene Therapy using non-hereditary cells. This section will also describe the purpose of the therapy, why it is different from other medical treatment and make the distiction between somatic-cell and germ-line gene therapy. Drs. Mahoney and Wieder have taken on this section of the GENERAL INFORMATION document.

The second section of the document will refer more specifically to the "Points to Consider" and is subtitled Governmental and Public Oversight. Attorney Judith Areen and I are working on this section.

The third section is being put together by Drs. Walters and Rich and will include possible anticipated concerns and adverse effects on the one hand, and on the other, some examples of the acceptability of human somatic-cell gene therapy.

A final section will list some references, a few articles and books for those who wish to study the subject further. This section will include an offer to send some NIH materials such as copies of the "Points to Consider", the Guidelines and the OPRR pamphlet, upon request.

Our working group plans to present a draft of the document to the Human Gene Therapy Subcommittee when it meets next on April 24th. When the Subcommittee has finalized the document, it will presented to the RAC for approval.

Anne R. Witherby
Public Representative and
Chairman, Working Group on a General Information Document.